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**PROCEEDINGS OF THE  
23<sup>TH</sup> NATIONAL CONGRESS  
OF THE  
“GRUPPO ITALIANO  
PER LO STUDIO  
DELLA NEUROMORFOLOGIA” G.I.S.N.**

*Cagliari, November 22-23, 2013  
University of Cagliari  
AULA MAGNA PALAZZO BELGRANO  
Via Università, 40 - Cagliari*

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C. Pellicciari

Dipartimento di Biologia e Biotecnologie  
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# *European Journal of Histochemistry*

## *a journal of functional cytology*

The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published until 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is published under the auspices of the University of Pavia and of the Ferrata Storti Foundation, Pavia, Italy.

The *European Journal of Histochemistry* is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC).

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Book Reviews, Views and Comments, concerning investigations performed with the aid of biophysical, biochemical, molecular-biological, enzymatic, immunohistochemical, cytometric, and image analysis techniques.

Areas of particular interest to the *European Journal of Histochemistry* include:

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# PROCEEDINGS OF THE 23<sup>TH</sup> NATIONAL CONGRESS OF THE “GRUPPO ITALIANO PER LO STUDIO DELLA NEUROMORFOLOGIA” G.I.S.N.

*The Italian Group for Neuromorphological Studies (Gruppo Italiano per lo Studio della Neuromorfologia, G.I.S.N.) is a scientific association promoted in 1990 by Prof. Damiano Zaccheo, eminent anatomist at the University of Genoa, together with Professors*

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*Since its foundation, the G.I.S.N. aimed to promote and develop scientific research in the morphology of the Nervous System, by gathering the research teams working in the field around Italy and organising scientific meetings, courses and seminars, both on scientific and technological issues.*

*Members of G.I.S.N. meet every second year at the prestigious Academy of Sciences of Bologna, and in the other years in different Italian seats in order to enlarge its visibility and the participation of new members, especially the youngest. It is this mixture of experienced researchers, who are requested to give the main lectures, and of young students enrolled in PhD programs, who present their scientific communications on the front line, that makes the meeting very special. In the course of more than twenty years, the members of G.I.S.N. grew together in science, collaboration and acquaintance. The meetings disseminated new seeds in the field of the Italian Anatomy, and represented a major appointment for Neuroanatomists of any age.*

*This year the meeting goes back to its origins, since the first one was held in Cagliari in 1991.*

*This volume gathers the scientific contributions to the meeting.*

*Gruppo Italiano per Lo studio della Neuromorfologia G.I.S.N.  
President, Marina Del Fiacco  
General Secretary, Alessandro Vercelli  
Treasurer, Daniele Tomassoni*

## MAIN LECTURES

### THE NEUROCHEMISTRY OF TOUCH RECEPTORS

J. Haseleu, S.G. Lechner, G.R. Lewin

*Molecular Physiology of Somatic Sensation Group, Max Delbrück Center for Molecular Medicine, Berlin, Germany*

Somatic sensation is overwhelmingly felt as the result of mechanical stimulation or movement of the body or its parts. Indeed almost all tissues of the body receive an innervation from the peripheral axons of mechanosensitive sensory neurons with their cell body in the dorsal root or trigeminal ganglia. The mechanisms and molecules used by sensory neurons to transform mechanical force into an electrical signal are poorly understood. The excitation of touch receptors is a two-step process characterized first by depolarization of receptor endings and second by an encoding event in which action potentials trains are initiated that signal the nature of the mechanical stimulus to the nervous system. We do not have a complete understanding of the molecules involved in this process, but it is clear that some molecules directly involved in this process should be localized to morphologically distinct nerve endings in the skin. Recently, we identified the potassium channel protein KCNQ4 as an essential for the normal encoding of sensory information by rapidly adapting hair follicle and Meissner's corpuscle receptors. The KCNQ4 protein is accordingly found to be localized only at the morphological endings of this specific class of receptors. Another protein that is involved directly in the transformation of sensory stimuli is the membrane protein STOML3. This protein is transported to peripheral terminals in a newly defined vesicle that we have termed the transducosome. In the absence of STOML3 the symptoms of neuropathic pain are dramatically attenuated and touch sensation is dramatically impaired. We have developed a new anatomical technique based on 2-photon microscopy to map the spinal projections of sensory afferents and their somatotopic organization in normal mice and mice with sensory deficits, like STOML3 mutant mice. My lecture will illustrate how new clearing and microscopy methods can illuminate the anatomy of the nervous system in ways that have not previously been attainable.

### NEUROPROTECTION IN NEUROTRAUMA: NOT LOST IN TRANSLATION?

A. Michael-Titus

*Centre for Neuroscience and Trauma, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, Whitechapel, London E1 2AT, United Kingdom*

Acute neurological injury in the central nervous system remains a major unmet need in neurology. Traumatic brain and spinal cord injury affect predominantly younger patients and are associated with high morbidity and significant health care costs. The lifelong debilitating consequences of central neurotrauma range from significant motor impairment to autonomic nervous system dysfunction, chronic pain, spasticity, depression, epilepsy and personality changes. The tissue injury processes triggered by trauma involve a variety of mechanisms: excitotoxicity, oxidation, neuroinflammation and metabolic compromise. Neuroprotection is a broad term that covers various strategies designed to protect the structure and function of the nervous system. In the context of neurotrauma, neuroprotective treatment could be delivered by the emergency and trauma team, in the immediate aftermath of injury. Much preclinical work has been devoted to the identification of neuroprotective agents, but the successful translation of neuroprotection proves difficult. The trials of neuroprotective agents carried out in the last three decades have been associated with repeated failure, although the preclinical data in experimental models were predicting therapeutic efficacy. The high rate of attrition in translation highlights the need to challenge many assumptions about the reaction of the nervous system to injury and degeneration. The very limited success in translation also suggests the need for new models and approaches. Examples such as progesterone, erythropoietin and minocycline are part of a revival of interest in neuroprotection and the promising results obtained with these agents in on-going trials support the validity of the concept. Optimised translational paths, the characterization of reliable and accessible biomarkers of injury and the development of imaging modalities which can cross the translational gap, will contribute to further refinement of the experimental protocols and an improved ability to select the most promising therapeutic targets for future trials. It is now realistic to hope that improved management of the acute phase of injury will have a significant positive impact on the quality of life and long-term prospects of neurotrauma patients.

## THE G.I.S.N. RESEARCH GROUPS

*In memoriam of Prof. Maria Luisa Lucchi*

### **A TRANSLATIONAL NEUROGASTROENTEROLOGY LAB: FROM CLINICAL TO SCIENCE...AND BACK**

R. De Giorgio

*Dept. of Medical and Surgical Sciences / Digestive Diseases and Internal Medicine, University of Bologna and St. Orsola-Malpighi Hospital, Bologna, Italy*

Until four decades ago, the term 'gastrointestinal motility' was thought adequate to describe the activity of scientists and clinicians who shared interests in gut motor activity. However, in the 80s and 90s, new advancements (e.g. in neuroscience, psychiatry, immunology, genetics and endocrinology) along with the discovery of novel and revolutionary experimental approaches led to a thorough remodeling of gastrointestinal motility. Since then the name neurogastroenterology was felt more appropriate in the scientific community and therefore adopted worldwide.

The group established about thirty years ago at the University Hospital St.Orsola-Malpighi in Bologna represents a clear example of integration between clinical and basic neurogastroenterology. The scope of this lab has been to investigate patients with functional gastrointestinal disorders ranging from commonly detectable conditions, e.g. irritable bowel syndrome and functional dyspepsia up to severe derangements of gut motor activity such as that underlying patients affected by achalasia, gastroparesis, chronic intestinal pseudo-obstruction (CIPO) and colonic inertia. The most recent advancements include genetic analysis of patients with syndromic forms of familial CIPO in an attempt to detect the mutations of gene(s) controlling the development, maintenance and survival of nerves, interstitial cells of Cajal (the enteric pacemakers) and smooth muscle cells. Data so far obtained indicated that *RAD21* mutations can play a pathogenetic role in severe gut dysmotility, i.e. CIPO. In line with these results, APOB a down-stream messenger of the RET cascade, which is regulated by *RAD21*, increased considerably in the sera of CIPO patients. A link between *RAD21* and APOB may have implications in the clinical setting. Other studies concerned a novel approach to investigate neurodegenerative disorders of the central nervous system which affect considerably the gastrointestinal tract. This is the case of Parkinson's disease (PD) and the analysis of the enteric plexi made possible through simple mucosal biopsies obtained from PD patients with severe constipation. The enteric neuronal analysis indicated neurochemical changes in the sub-mucosal neurons likely contributing to altered gut secretomotor function in constipated PD patients. In conclusion, the neurogastroenterology lab provides a valuable translational view which is expected to improve management and treatment of patients with functional gastrointestinal disorders.

Supported by Fondazione Del Monte di Bologna e Ravenna.

## NEUROMORPHOLOGY AND INNOVATION

### **BRAIN IMAGING: FROM MORPHOLOGY TO FUNCTIONAL CONNECTIVITY; THE ROLE OF MAGNETIC RESONANCE**

L. Barberini<sup>1</sup>, M. Frascini<sup>2</sup>, M. Puligheddu<sup>1</sup>, F. Marrosu<sup>1</sup>

<sup>1</sup>Dept. of Public Health, Molecular and Clinical Medicine, University of Cagliari, Cagliari; <sup>2</sup>Dept. of Electrical and Electronic Engineering, University of Cagliari, Cagliari, Italy

The recent rising and impact of the neurological diseases on the modern human society justifies the worldwide increase of interest for the dramatic innovation in those diagnostic techniques aimed at better understanding the wiring and wiring of the human brain. Although several methods are currently used, the fascination of the functional magnetic resonance (fMRI) has taken the scene in most recent years, thanks the diffusion of Magnetic Resonance (from which the fMRI derives) currently considered the golden standard in non-invasive diagnostic procedures in neurology as well as in other medical fields. Technically (and briefly) as no ionizing radiation are used, in fMRI image "contrast" of brain areas is realized mostly by the oxygenizing-deoxygenizing vascular activity correlated with neuronal work (BOLD effect). Although the detection is quite approximate (e.g. neither inhibitory nor excitatory neurons are singled out), the area involved is visualized. This unique characteristic allows for a wealth of studies aimed at correlating brain areas with their functions (and dysfunctions). Moreover, this real breakthrough in imaging procedures has also induced theoretical shortcuts and "cheap" utilizations (e.g. the use of fMRI as technical support in tailoring commercial advices). Nonetheless, fMRI has increasingly become "the tool par excellence" not only in exploiting brain functionality but also in correlating brain areas during their activity (brain networking) and even in supporting studies on brain-mind interface. In the present work we present a multimodal application of fMRI, namely by co-registrate EEG signals in a case of musicogenic epilepsy which supports the hypothesis that, although impaired by an epileptic event, the activity of the brain "runs business as usual", while the second case deals with the unconscious deception operated by the brain during an hysterical condition: in this rare case we can detect, instead of the parietal area, the activation of a frontal area (supposed to be involved in decision making) while the limb affected by hysterical anesthesia is painfully stimulated. This case really seems to bridge the once considered psychic symptom (Freudian "conversion") with its organic support.



## HISTORY OF NEUROMORPHOLOGY

### ART AND ANATOMY IN THE WAXES OF SUSINI-BOI AT THE UNIVERSITY OF CAGLIARI

A. Riva

*Museum of Anatomical Waxes and Dept. of Biomedical Sciences, University of Cagliari, Italy*

The waxes of Cagliari, having been manufactured between 1803-1805, are later than those of the great collections of the La Specola in Florence and of the Josephinum in Vienna, and represent a work of the maturity of Clemente Susini (1754-1814), the chief modeler of the La Specola museum. The dissections reproduced by Susini were performed by Francesco Antonio Boi (1767-1865), the anatomist from the University of Cagliari who had been sent on purpose to Florence by the Viceroy of Sardinia Carlo Felice of Savoy (1765-1831). The models, which arrived in Cagliari in 1806 contained in 23 show-cases, are attached to 23 wooden tables that still bear the original tag with date and Susini's signature. The latter is a distinctive character not present in the other collections that were known as Fontana's opus. Moreover, they were made when Susini was able to fully express his own artistic view, being eventually free from the somewhat oppressing influence of his great master Felice Fontana, who in that time was no more the manager of La Specola. By express order of Carlo Felice, the models to be made for Cagliari, which consist of a special mix of waxes able to resist the hot climate of south Sardinia, had to be unique and, thus, at variance with those of La Specola, no copies of them are seen in other collections. No standing figures are present. Susini's more mature style is particularly evident in the faces, that are realistic portraits and veritable masterpieces of figurative art, in the skilful and harmonious use of colours, and in the perfect rendering of anatomical details. On the other hand, possibly thanks to Francesco Antonio Boi, a few mistakes and omissions seen in the collections of Florentine waxes of La Specola, Vienna, and Bologna, such as the presence of lymphatics in brain, are absent here.

## SESSION I NEURAL PLASTICITY AND DEVELOPMENT

### EXPRESSION ANALYSIS OF PLURIPOTENCY-ASSOCIATED GENES IN HUMAN FETAL CORTICAL AND STRIATAL NEURAL STEM CELLS DURING DIFFERENTIATION

D. Massa<sup>1</sup>, R. Pillai<sup>1</sup>, E. Monni<sup>2</sup>, Z. Kokaia<sup>2</sup>, A. Diana<sup>1</sup>

<sup>1</sup>Dept. Biomedical Sciences, Section Cytomorphology, Lab. Neurogenesis and Neurogenesis, University of Cagliari, Monserrato, Italy; <sup>2</sup>Lund Stem Cell Center, Lab. Stem Cells and Restorative Neurology, University of Lund, Lund, Sweden

Human neurospheres are free-floating spherical clusters generated from a single neural stem cell and comprising cells at different stages of maturation in the neuronal and glial lineages. Although recent findings have disproved the original idea of clonally derived neurospheres according to the paradigm of one stem cell → one neurosphere, they still represent a valid model for growing neural stem cell cultures *in vitro*. Since in the field of developmental biology, compelling evidence for a network of activity has been tributed to transcriptional molecules such as Oct4, Nanog and nestin, during neurogenesis, the choice between enhancement versus suppression of transcriptional modulation of some pluripotency and stem-associated genes would determine the balance between self-renewal neural stem cells (NSC) and immature neuronal phenotypes.

Thus, by means of immunocytochemistry and RT-PCR methods, our study aimed to address the question whether and to what extent mRNA and protein profiles are expressed in human fetal neurospheres obtained from cortical and striatal brain regions. Morphological and biochemical analysis was carried out both in expansion (undifferentiated cells) and differentiation conditions monitored after 1 and 4 weeks *in vitro* culturing. Our results clearly demonstrated the sustained presence of opposite signals: strong downregulation of Oct4 and Nanog genes in cortical differentiating cells and significant up-regulation for nestin gene both in cortical and striatal differentiating cells. Notably, by immunostaining techniques, Oct4 and Nanog protein expression were detected not only in the nuclear compartment but also in the cytoplasmic domain followed by their rapid turnover (immediately after 1 week). In addition, in the course of the differentiation process, dissociated neurospheres displayed unexpected number of nestin positive cells accompanied by a constant level of staining intensity. In conclusion, the present study provides new insights into brain region related features in terms of Oct4, Nanog and nestin expression both at cellular and molecular level.

## IMMUNOHISTOCHEMICAL STUDY ON THE EFFECTS OF MORPHINE ON ERK PHOSPHORYLATION IN THE NUCLEUS ACCUMBENS

M. Rosas<sup>1</sup>, E. Acquas<sup>1,2,3</sup>

<sup>1</sup>Dept. of Life and Environmental Sciences - Pharmaceutical, Pharmacological and Nutraceutical Sciences Section; <sup>2</sup>Centre of Excellence on Neurobiology of Addiction and <sup>3</sup>National Institute of Neuroscience - INN, University of Cagliari, Cagliari, Italy

A critical role of phosphorylated Extracellular signal Regulated Kinase (pERK) in the actions of addictive drugs has recently emerged. However, some controversial findings still remain on the effects of morphine on ERK phosphorylation in the nucleus accumbens (Acb). The present study was devoted to investigate this issue by comparatively assessing the ability of morphine to activate ERK phosphorylation in the Acb shell (AcbSh) and core (AcbC) of Sprague-Dawley and Wistar rats and on CD-1 and C57BL6J mice. To this end, 20 or 40 minutes after morphine (1 or 5 mg/kg) administration, pERK expression was measured as % pERK-positive neurons/area by immunohistochemistry. In rats, morphine decreased % pERK-positive neurons in the AcbSh and AcbC. Notably, pre-treatment with naltrexone (1.5 mg/kg) ( $\mu$  opioid receptor antagonist) but not with SCH 39166 (50  $\mu$ g/kg) (dopamine D1 receptor antagonist) prevented these reductions. In mice, morphine, preferentially in the AcbSh compared to the AcbC, increased % pERK-positive neurons/area and these effects were prevented by both naltrexone and SCH 39166. These observations confirm the differential effects of morphine on ERK phosphorylation in rats' and mice' Acb. In addition, these results indicate that  $\mu$ - and D1-mediated processes are differentially involved in the mechanism(s) by which morphine affects pERK expression in the Acb and call for further experiments to elucidate the significance of the property of morphine to either increase and decrease pERK expression in rodents' AcbSh and AcbC.

## CHANGES ON NEURONAL PLASTICITY IN PHYSIOLOGICAL (POSTPARTUM) AND STRESSED CONDITIONS

M.C. Mostallino<sup>1</sup>, P.P. Secci<sup>2</sup>, V.M. Melis<sup>2</sup>, G. Biggio<sup>1,2</sup>

<sup>1</sup>Institute of Neuroscience, National Research Council (CNR), Cagliari; <sup>2</sup>Dept. of Life and Environmental Sciences, Section of Neuroscience, University of Cagliari, Cagliari, Italy.

Neural plasticity, also known as neuroplasticity, is the capability of neurons to change the structure, function and organization of neurons in response to new experiences. It specifically refers to strengthening or weakening nerve connections or adding new nerve cells based on environmental stimuli. These processes are responsible for physiological changes, learning and the formation of appropriate responses to external event. Neural plasticity is among the most important aspects of the field of modern neuroscience and its study is leading to a better understanding of brain development. In this work the neuroplasticity, in particularly the density of dendritic spines and neurogenesis, was studied after a prolonged stress, such as maternal separation. Our studies have shown an increased density of dendritic spines and neurogenesis after delivery; in contrast, a reduction of number of dendritic spines and neurogenesis was observed after a prolonged stress. These results demonstrate that physiological changes or social environments can have significant effects on neuronal plasticity.

## PHENOTYPIC CHARACTERIZATION AND NEURAL PLASTICITY OF OLFACTORY ENSHEATHING CELLS

R. Pellitteri<sup>1</sup>, P. Bossolasco<sup>2</sup>, L. Cova<sup>2</sup>, V. Silani<sup>2,3</sup>, D. Zaccheo<sup>4</sup>

<sup>1</sup>Inst. of Neurological Sciences, CNR, Section of Catania, Catania; <sup>2</sup>Dept. Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan; <sup>3</sup>Dept. Pathophysiology and Transplantation - "Dino Ferrari" Centre, Università degli Studi di Milano, Milan; <sup>4</sup>Dept. Experimental Medicine, section of Human Anatomy, University of Genoa, Genoa, Italy.

Olfactory Ensheathing Cells (OECs) show a peculiar plasticity and represent a unique population of glial cells that, in the olfactory system, support the continuous neuronal turn-over and sheathe olfactory axons. They also share antigenic and morphological characteristics both of astrocytes and Schwann Cells. *In vitro*, as source of neurotrophic factors (GFs), OECs promote axonal growth; moreover, *in vivo* they form myelin, promoting remyelination of damaged axons. In the last two decades, OECs have attracted considerable interest for their characteristics, emerging as possible candidates for transplantations to promote axonal regeneration after spinal cord injury. These findings have stimulated us to better investigate the potential of OECs for regeneration and functional recovery of damaged Central Nervous System (CNS). In this study, OECs were isolated from 1-day old mouse pups olfactory bulbs and cultured in DMEM/FBS. After reaching confluence, OECs were trypsinized and subcultured in multi-well plates and a characterization was performed both by flow cytometry and immunocytochemistry for the following markers: vimentin, S100, nestin, Glial Fibrillary Acidic Protein (GFAP), Myelin, Neural Cell Adhesion Molecule (NCAM), low-affinity nerve growth factor receptor p75, Microtubule Associated Protein-2 (MAP-2) and Protein Gene Product (PGP 9.5). In order to study the modulation of these markers and OEC plasticity, cells were also grown in different culture conditions: standard or serum-free media with/without Growth Factors (GFs), such as basic Fibroblast Growth Factor (bFGF) and Glial Derived Neurotrophic Factor (GDNF). Basal apoptosis was evaluated by annexin and propidium iodide analysis as well as after exposition to the neurotoxin 6-hydroxydopamine (6-OHDA). Murine neural stem cells (NSCs) were used as control. Our results showed: 1) a reduced cell viability and marker expression both by immunocytochemistry and flow cytometry coupled to the loss of OEC usual morphology in serum-free medium; 2) GF addition influenced positively OEC viability and marker expression both in complete medium and serum-free DMEM; 3) no apoptosis and death of OECs in comparison to the sensitive NSCs after exposure to the 6-OHDA (100mM) for 6 hours and 12 h were observed. These peculiar properties of OECs might render them as useful potential clinical agents being able to support injured CNS.

## EVIDENCE OF DIFFERENTIAL EXPRESSION RELATED TO THYROSINE HYDROXYLASE GENES IN CHICK (*GALLUS GALLUS*) BRAIN STEM DURING EMBRYO DEVELOPMENT

A. Barca, A. Fonzino, A. Romano, T. Verri, F. De Nuccio, G. Nicolardi, D.D. Lofrumento

Dept. of Biological and Environmental Sciences and Technologies – Di.S.Te.B.A., University of Salento, Lecce, Italy

By affecting efficiency of DOPA synthesis, tyrosine hydroxylase (TH) is the effective modulator of the limiting step of the biosynthetic pathways of catecholamines (dopamine, noradrenaline and adrenaline), highly conserved in vertebrate species. Catecholamines are involved in many physiological functions in the central and peripheral nervous systems as well as in the endocrine system, thus regulation of TH expression and activity are crucial for neuronal and hormonal functions that involve the entire dopaminergic, noradrenergic and adrenergic systems. TH-related expression is due to two non-allelic genes, called TH1 and TH2, reported in almost all vertebrates except placental mammalian, which have lost TH2 gene during evolution. TH-related genes have crucial ontogenetic roles, being linked to pathological onsets during embryo development. Here, we show the expression analysis of TH-related transcripts in brain stem of *gallus gallus*, as a key model of vertebrate central nervous system development. By real time RT-PCR assays, we assessed that TH1 and TH2 mRNAs show progressive increase during embryo development (from 8 to 21 days post fertilization) with differential trend. Moreover, a substantially different regulatory switch of expression was shown for the two genes when passing to the adult developmental phase. According to what stated in teleost fish, different expression patterns suggest different mechanism of transcriptional regulation related to potentially differing roles during development for TH1 and TH2 genes: based on our comparative results, TH1 mRNA expression in *gallus gallus* brain increases gradually during development reaching significantly high post-embryonic levels, whereas the TH2 mRNA seems to be more specifically linked to embryogenesis of vertebrate brain stem.

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## NEUROSCIENCE FOR LUNCH

### THEME 1 CHEMICAL SENSES

#### PALPAL RECEPTORS OF THE OLIVE FLY *BACTROCERA OLEAE* PLAY A KEY ROLE IN FORAGING BEHAVIOR AND HOST FINDING

A. Liscia<sup>1</sup>, P. Sacchetti<sup>2</sup>, M.D. Setzu<sup>3</sup>, S. Poddighe<sup>3</sup>, F. De Rose<sup>1</sup>, A. Belcari<sup>2</sup>

<sup>1</sup>Dept. of Life and Environmental Sciences, Section of Neurosciences, University of Cagliari, University Campus Monserrato (CA); <sup>2</sup>Dept. of Agrifood Production and Environmental Sciences, Section of Plant Protection, University of Florence; <sup>3</sup>Dept. of Biomedical Sciences, University of Cagliari, University Campus Monserrato (CA) Italy

The olive fly *Bactrocera oleae* (Rossi) is a serious pest of olives in several countries in the Mediterranean basin causing important losses in the oil industry. The olive fly is known to respond behaviorally to volatile compounds present in its habitat and, more specifically, to those released from its host plant that play an important role in guiding the oviposition behavior of gravid females. A recent research by Liscia *et al.* (2013) on a laboratory strain of *B. oleae* demonstrated that volatiles released from a bacterial filtrate (obtained culturing *Pseudomonas putida*) are mainly detected by palpal olfactory receptors rather than the antennal sensilla. On the contrary,  $\alpha$ -pinene, a key compound in triggering the oviposition (Scarpati *et al.*, 1993) that is emitted by leaves and half-ripe olives is mainly detected by male insects with the antennal receptors. On these bases, this study has been aimed to further investigate the role of maxillary palps in detecting food and oviposition sites in wild *B. oleae* adults obtained from pupae collected in different areas of olive orchards in Sardinia. Electrophysiological (EAG and EpG) and behavioral bioassays (Y-tube olfactometer and wind tunnel) were performed to test bacterial filtrate volatiles and some host plant ( $\alpha$ -pinene) and food sources (acetic acid) related compounds. Dose-response relationships and differences in sensitivity related to insect sex and physiological condition were identified. Responses were compared to those obtained in lab insects. The results obtained in wild insects confirmed that palpi have a higher sensitivity to bacterial filtrate than the antennae. Otherwise, the EpG recorded in mated females in response to  $\alpha$ -pinene and acetic acid, showed a lower threshold and a greater signal amplitude than those recorded in lab insects both in the palpi and the antennae. Electrophysiological results are complementary with the behavioral ones. In conclusion, the maxillary palp olfactory receptors play a primary role in the short-range detection of chemical cues from host plant and epiphytic bacteria. These findings open new perspectives for improving olive fly control strategies.

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**PAPILIO HOSPITON LARVAE DISCRIMINATE BITTER AND SWEET STIMULI BY THE "LABELED LINES" CODING MODALITY**

G. Sollai, I. Tomassini Barbarossa, C. Masala, P. Solari, R. Crnjar  
*Dept. of Biomedical Sciences, Section of Physiology, University of Cagliari, Italy*

In herbivorous animals, food selection may depend on sensitivity to specific chemical stimuli as well as to secondary metabolites (bitter compounds) and sugars (phagostimulant compounds). Bitter compounds are noxious, unpalatable or both and evoke an aversive feeding response. Instead, sugars and sugar alcohols play a critical role in determining and enhancing the palatability of foods.

It is suggested that whether one animal eats or not a given food depends on the total "sensory impression" which results from the integrated response to phagostimulant and deterrent compounds at level of the CNS. The coding mechanisms generally proposed for neural processing of sensory information are: 1) a "labeled lines" code, by which the stimulus identity is represented by the activity in a subset of afferent taste neurons, and 2) an "across fiber patterning" code, where tastant identity is represented by activity across large populations of afferent gustatory neurons.

We investigated the discriminating modalities in the larvae of lepidopterous species (*Papilio hospiton*), that has the advantage of possessing a limited number of gustatory receptor neurons, the axons of which project directly to the CNS. The spike activity of the medial and lateral maxillary styloconic taste sensilla was recorded following stimulation with several carbohydrates, nicotine and NaCl. With the aim of evaluating the discrimination capability between different compounds and the coding mechanism used, we calculated the neural discrimination (d) and the labeled line (l) indexes. The results show that *P. hospiton* is capable of discriminating bitter from sugar tastants and different bitter and sugar compounds from one another: the neural code principally used is of the "labeled lines" type.

**THEME 2  
PERIPHERAL NEUROPATHY AND REPAIR**

**STUDY OF THE CALCITONIN GENE-RELATED PEPTIDE-POSITIVE INTRAEPIDERMAL NERVE FIBERS IN A RAT MODEL OF BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY**

M. Boi<sup>1</sup>, M. Quartu<sup>1</sup>, M.P. Serra<sup>1</sup>, L. Poddighe<sup>1</sup>, T. Melis<sup>1</sup>, C. Picci<sup>1</sup>, M. Del Fiacco<sup>1</sup>, C. Meregalli<sup>2</sup>, A. Canta<sup>2</sup>, A. Chiorazzi<sup>2</sup>, B. Sala<sup>2</sup>, N. Oggioni<sup>2</sup>, G. Cavaletti<sup>2</sup>, V. Carozzi<sup>2</sup>

<sup>1</sup>Dept. of Biomedical Sciences, University of Cagliari, 09042 Monserrato, Italy; <sup>2</sup>Dept. of Surgery and Interdisciplinary Medicine, University of Milan-Bicocca, Monza (MB), Italy

Bortezomib (BTZ), a selective proteasome inhibitor, is a potent antineoplastic drug to treat multiple myeloma. In clinical practice, the most significant side effect of its administration, is a peripheral painful sensory neuropathy (PPN), characterized by numbness and tingling in a distal stocking-glove distribution and distal paresthesia, which is generally associated with impairment of Adelta and C type primary afferent fibers and whose treatment is symptomatic and scarcely effective. To evaluate if BTZ-induced sensory deficits are associated to loss of epidermal nerve fibers, as observed in other PPN conditions, here we examine the effect of a well-established chronic BTZ-treatment schedule on the number of intraepidermal nerve fibers (IENF) in a rat model of BTZ-induced PPN and compare the outcome of chronic BTZ-treatment followed by two-weeks of follow up with that of animals that received a subsequent 2-weeks treatment period with Gabapentin (Gaba) and buprenorphine (Bupre), two analgesic drugs commonly used in clinical practice.

Female Wistar rats were treated with BTZ 0.20 mg/kg, 3 times a week for 8 weeks (i.v.). Then analgesic were administered according to the following curative schedule: Gaba (100 mg/kg, daily, p.o.), Bupre (28,8 µg/kg, daily, s.c.). At the end of treatments, the hindlimb footpad skin was examined by immunohistochemistry for the pan-neuronal marker PGP9.5 and calcitonin gene-related peptide (CGRP), a neuropeptide involved in pain perception and neurotransmission.

Labelled nerve fibers were distributed in the reticular and papillary dermis and within the epidermal lining. Quantitative evaluation of IENF showed that the mean linear density of CGRP-labelled IENF normalized by that of the PGP9.5-labelled ones shows a statistically significant decrease in BTZ-treated rats. None of the analgesics used in this study appeared to reverse the BTZ-induced loss of IENF.

Results obtained suggest that the CGRP-positive innervation is likely involved in the persistence of BTZ-induced pain and that detectability of CGRP-positive IENF appears not to be related to the effectiveness of the selected analgesics.

## IMIDAZOLINE RECEPTOR 2 IS AN EFFECTIVE TARGET FOR NEUROPATHIC PAIN IN A MURINE MODEL OF BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY

V.A. Carozzi<sup>1</sup>, C. Meregalli<sup>1</sup>, A. Canta<sup>1</sup>, A. Chiorazzi<sup>1</sup>, B. Sala<sup>1</sup>, N. Oggioni<sup>1</sup>, M. Lanza<sup>2</sup>, M. Quartu<sup>3</sup>, M.P. Serra<sup>3</sup>, L. Poddighe<sup>3</sup>, C. Picci<sup>3</sup>, M. Boi<sup>3</sup>, T. Melis<sup>3</sup>, M. Del Fiacco<sup>3</sup>, G. Caselli<sup>2</sup>, G. Cavaletti<sup>1</sup>

<sup>1</sup>Dept. of Surgery and Interdisciplinary Medicine, University of Milan-Bicocca, Monza (MB); <sup>2</sup>Pharmacology and Toxicology Department, Rottapharm Madaus Research Center, Monza (MB); <sup>3</sup>Dept. of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Monserrato (CA), Italy

Bortezomib (BTZ) is a proteasome inhibitor used as first-line therapy for multiple myeloma. However, its administration induces the development of severe painful peripheral neuropathy (PPN). This painful condition is an important medical need since the available treatments are actually ineffective. We recently described a mice model of PPN that shares most of the conditions found in patients treated chronically with BTZ (Carozzi et al., 2013). In fact, BTZ determines dysfunction of all fiber types in sensory nerves and, at least in mice, alters the electrical activity of the spinal dorsal horn neurons. This alteration of the basal electrophysiological activity induces also relevant changes in the central nociceptive transmission. In this work we characterize the neuroprotective effects of an imidazoline receptor 2 ligand (CR4056) able to allosterically inhibit the activity of monoamine oxidase-A, a key enzyme in the regulation of neuropathic pain.

Wistar rats were treated with BTZ 0.20 mg/kg, 3 times a week for 8 weeks (i.v.). Then CR4056 was orally administered in a curative schedule at 6 mg/kg, once a day, for 2 weeks. Gabapentin (100 mg/kg, daily, p.o.) and buprenorphine (28,8 µg/kg, daily, s.c.) were used as internal analgesic standards. At the end of both BTZ and analgesic treatments, we measured the caudal and sciatic nerve conduction velocity (NCV), the morphological/morphometrical alterations in the caudal nerve and the neuropathic pain development.

BTZ treatment induced a significant impairment of sensory, but not motor NCV, slight hyperalgesia, significant mechanical allodynia and clearing of myelinated fibers in the caudal nerves. After two weeks of follow up animals did not spontaneously recover functional, morphological and behavioral abnormalities while the 2 weeks-treatment with CR4056 (but not with gabapentine and buprenorphine) significantly resolved BTZ-induced mechanical allodynia.

Results obtained show that CR4056 produces a marked analgesic effects against BTZ-induced neuropathic pain without signs of tolerance.

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## EXPRESSION OF TRPV1 AND CGRP IN SPINAL PRIMARY AFFERENT NEURONS IN A RAT MODEL OF BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY TREATED WITH ANALGESICS

L. Poddighe<sup>1</sup>, M. Quartu<sup>1</sup>, M.P. Serra<sup>1</sup>, M. Boi<sup>1</sup>, T. Melis<sup>1</sup>, C. Picci<sup>1</sup>, M. Del Fiacco<sup>1</sup>, C. Meregalli<sup>2</sup>, A. Canta<sup>2</sup>, A. Chiorazzi<sup>2</sup>, B. Sala<sup>2</sup>, N. Oggioni<sup>2</sup>, G. Cavaletti<sup>2</sup>, V. Carozzi<sup>1</sup>

<sup>1</sup>Dept. of Biomedical Sciences, University of Cagliari, Monserrato, Italy; <sup>2</sup>Dept. of Surgery and Interdisciplinary Medicine, University of Milan-Bicocca, Monza, Italy

Bortezomib (BTZ), a selective proteasome inhibitor, is an antitumor drug used to treat multiple myeloma. As a side effect, BTZ determines a painful peripheral neuropathy (PPN), often refractory to management. Chronic BTZ administration in female Wistar rats induced a PPN in which the development of mechanical allodynia is associated to an increase in the expression of the transient receptor potential vanilloid type 1 (TRPV1) receptor and calcitonin-gene related peptide (CGRP) in the dorsal root ganglia (DRG) and spinal cord dorsal horn. In this study we examine the possible role of two standard analgesics, Gabapentin (Gaba) and buprenorphin (Bupre), in modulating the expression of TRPV1 and CGRP in the DRG and spinal cord of rats chronically treated with BTZ. To this aim, female Wistar rats were treated with BTZ 0.20 mg/kg, 3 times a week for 8 weeks (i.v.). Then Gaba (100 mg/kg, daily, p.o.) and Bupre (28,8 µg/kg, daily, s.c.) were administered in a curative schedule for 2 weeks. The expression of TRPV1 and CGRP, a neuropeptide involved in nociceptive neurotransmission, was examined in L4-L5 DRG and spinal cord segments by means of western blot (WB) and immunohistochemistry. WB analysis showed a statistically significant decrease of protein levels in DRG of BTZ-treated rats compared to controls, whereas did not show any change in the spinal cord. Instead, chronic BTZ treatment followed by a 2-weeks follow-up period affected TRPV1 expression by inducing an increase in the proportion of TRPV1-like immunoreactive (LI) DRG neurons, whereas the percentage of CGRP-LI neurons did not change. Labeled perikarya were mostly of small- and medium-size. No BTZ-induced changes in immunolabeling were appreciable in the dorsal horn of the spinal cord. Treatment with Gaba and Bupre reduced levels of TRPV1 protein in spinal cord homogenates, whereas it had no effect on DRG protein levels. None of the analgesics appeared to reverse in a statistically significant way the BTZ-induced increase of labelled DRG neurons, nor induced any change in the detectability of positive innervation in the spinal cord dorsal horn.

## PERIPHERAL NERVE REGENERATION: A COMPARATIVE LIGHT – ELECTRON MICROSCOPIC STUDY



S. Geuna<sup>1</sup>, R. Giulia<sup>2</sup>, S.B. Jager<sup>2</sup>, C.B. Vaegter<sup>2</sup>, S. Raimondo<sup>1</sup>, M.G. Robecchi<sup>1</sup>

<sup>1</sup>Dept. of Clinical and Biological Sciences, University of Turin, Orbassano (TO), Italy; Neuroscience Institute of the "Cavalieri Ottolenghi" Foundation (NICO), University of Turin, Orbassano (TO), Italy; <sup>2</sup>The Lundbeck Foundation Research Center MIND, Department of Medical Biochemistry, Aarhus University, Denmark

Injuries to the peripheral nervous system occur frequently and are a major source of disabilities, resulting in partial or total loss of motor and sensory functions. Despite it is well known that the peripheral nervous system has the capacity to regenerate and re-innervate denervated target organs, clinical and experimental evidences show that the regeneration is usually far from satisfactory, especially after severe injuries.

Quantitative estimation of myelinated nerve fiber number, together with fiber size parameters, is one of the most important tools for objectivity in the description of morphologic changes during nerve regeneration, since it correlates better with the functional outcome of nerve recovery.

In this study we used a design-based stereology method to evaluate the regenerative process in two experimental groups: crush injury and autograft repair. As control group, uninjured nerves were used. Samples were embedded in resin and morphometric counting were done with both light and electron microscopy.

Results showed a significant difference in myelinated fiber number between LM and EM, especially after the regenerative process, where LM morphometry underestimates the number of fibers, due to the large number of very small axons that can be detected only in EM.

This comparative study shows that the integration of data obtained in LM with those obtained in EM is fundamental in revealing structures (very small myelinated fibers and unmyelinated fibers) that cannot be detected otherwise. Moreover, the difference in total number of myelinated fibers between LM and EM must be considered in data analysis to ensure accurate interpretation of the results.

## THEME 3 NEUROINFLAMMATION AND NEUROPROTECTION

### EFFECTS OF *WITHANIA SOMNIFERA* EXTRACT ON PAIN BEHAVIOUR AND SPINAL CORD C-FOS EXPRESSION AFTER FORMALIN-INDUCED NOCICEPTION IN MICE

M.A. Casu<sup>1</sup>, A. Orrù<sup>1</sup>, G. Casu<sup>1</sup>, S. Tambaro<sup>2</sup>, G. Marchese<sup>1</sup>, S. Ruiu<sup>1</sup>

<sup>1</sup>CNR, Institute of Translational Pharmacology, UOS of Cagliari, Scientific and Technological Park of Sardinia POLARIS, Loc. Piscinamanna, Pula, Cagliari; <sup>2</sup>Neuroscienze Pharmaness Scientific and Technological Park of Sardinia POLARIS, Loc. Piscinamanna, Pula, Cagliari, Italy

*Withania somnifera* Dunal (WS, family: Solanaceae) is a safe medicinal plant commonly used in Ayurvedic medicine to treat several diseases. Previous studies demonstrated that WS prevented the development of tolerance to the analgesic effect of morphine and suggested its potential anti-inflammatory properties. We recently found in mice that WS extract (WSE) prolonged morphine-induced anti-nociception and prevented the emergence of morphine-induced hyperalgesia although devoid of *per se* anti-nociceptive activity.

To investigate further the potential role of WS in the modulation of pain, we evaluated the ability of WSE to modulate mice's anti-nociceptive responses in the formalin test. c-FOS protein expression in L4-L5 spinal dorsal horn was also analysed as a marker of neuronal activation following noxious stimulation.

Male CD1 mice were pre-treated with 0, 100, 150 or 200 mg/Kg WSE (i.p.) 30 min before Formalin injection (20  $\mu$ l of 5% diluted in saline) into the left hind paw. The time spent licking the formalin-injected paw was recorded for 45 min. Phases were defined as follows: Phase I (0–5 min), Phase II (10–45 min). Fos-immunoreactive (Fos-IR) spinal neurons were stained according to a standard avidin–biotin–peroxidase technique using a rabbit polyclonal antibody against c-Fos protein and a commercial ABC kit. Fos-IR neurons were visualized with 3,3'-diaminobenzidine.

WSE was devoid of anti-nociceptive activity in the first acute phase of the formalin test, but interestingly, it reduced in a dose-dependent manner the inflammatory response during the late phase. Consistently, WSE significantly decreased formalin-induced Fos-IR in a dose-dependent manner in particular in the superficial layers.

Together, the results confirm the potential anti-inflammatory activity of *Withania somnifera* extract. The mechanism of action WSE-induced anti-inflammatory activity is under investigation.

### DOWN-REGULATION OF INFLAMMATORY MARKERS BY CONJUGATED LINOLEIC ACID (CLA) ISOMERS IN HUMAN CULTURED ASTROCYTES.

R. Pillai, F. Saba, A. Sirigu, L. Cordeddu, G. Carta, E. Murru, S. Banni, V. Sogos

Dept. of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Italy

Astrocytes represent the major glial cells in the central nervous system (CNS). After a damage or in various human diseases, astrocytes acquire reactive phenotype associated with the upregulation of molecule normally present in low levels in brain. Inflammation is modulated by different fatty acid derived molecules such as eicosanoids. In particular those from arachidonic acid are known to promote inflammation in different tissues. Conjugated linoleic acid (CLA) consists of a group of isomers of

linoleic acid that are found in food, such as meat and dairy products from cows and sheeps. CLA exerts a variety of biological activities including modulation of lipid metabolism and alteration of eicosanoid formation. However, no data are available whether this occurs in brain cells. In this study we aimed at evaluating whether CLA isomers are able to perturb fatty acid profile and hence the expression of pro-inflammatory molecules in human astrocytes.

Cultured astrocytes were treated for six days with 100  $\mu$ M fatty acids (c9,t11 CLA or t10,c12 CLA or oleic acid). Only t10,c12 CLA isomer induced a significant decrease of arachidonic acid and increased the ratio DHA/EPA, an indirect evidence of peroxisome proliferator activated receptor (PPAR)  $\alpha$  activation. PPARs are fatty acid receptors that regulate the expression of genes involved in immune function. Inhibition of TNF- $\alpha$ , IL-1 and RANTES expression was observed in astrocytes treated with c9,t11 CLA and t10,c12 CLA, while IL-1 $\alpha$  decreased only with t10,c12 CLA.

Our data demonstrate that CLA may weaken neuroinflammation by reducing the pro-inflammatory property of astrocytes. Thus, CLA may result in an adjuvant dietary component that could help to modulate neuroinflammatory mediators contributing to brain homeostasis.

### PROTECTIVE EFFECT OF CHOLINE ALPHOSCERATE ON INFLAMMATORY MARKERS IN CEREBROVASCULAR MORPHOLOGY OF SPONTANEOUSLY HYPERTENSIVE RATS

S.K. Tayebati<sup>1</sup>, D. Tomassoni<sup>2</sup>, I.E. Nwankwo<sup>1</sup>, F. Amenta<sup>1</sup>

<sup>1</sup>School of Medicinal Sciences and Health Products and <sup>2</sup>School of Biosciences and Biotechnology, University of Camerino, Italy

The effect of cholinergic precursors on choline availability and acetylcholine synthesis/release is established. Probably, these mentioned effects may counter cognitive impairment occurring in adult-onset dementia disorders. Choline alphoscerate (alpha-glycerol-phosphoryl-choline, GPC) is among cholinergic precursors the most effective in enhancing acetylcholine biosynthesis and release in animal models.

On the other hand, chronic brain vascular injury is a severe risk factor of cerebral dysfunction. White matter lesions represent the most important early consequences of cerebrovascular impairment. Cerebral hypoperfusion, which can induce small vessel disease (SVD), is closely linked to the development of white matter injury. Brain hypoperfusion and white matter lesions correlate with the development of cognitive impairment in Alzheimer's disease (AD) or vascular dementia (VaD).

The present study has assessed if long term treatment with GPC has a cerebroprotective effect on brain injury of vascular origin.

Analysis was made on spontaneously hypertensive rats (SHR). They were used as animal model of brain vascular injury. Male SHR aged 32 weeks and age-matched normotensive Wistar-Kyoto (WKY) rats were treated for 4 weeks with GPC (150 mg/kg/day) or vehicle. On pial and intracerebral arteries of different brain areas, vascular astrocytes, blood brain barrier (BBB) and endothelial markers were assessed by neuromorphological and immunohistochemical techniques associated with quantitative analysis.

No significant changes in the size of perivascular astrocytes were observed in SHR compared to WKY, whereas the expression of the BBB marker aquaporin-4 decreased in SHR. This phenomenon was countered by GPC treatment. Endothelial markers and vascular adhesion molecules (ICAM, VCAM, PECAM, and P-selectine) expression were not homogeneously affected by hypertension in both pial and intracerebral vessels.

The observation that treatment with GPC reversed cerebral microanatomical changes occurring in SHR is consistent with data of clinical trials reporting an improvement of cognitive function in subjects suffering from cerebrovascular disorders.

These preclinical data suggest a re-evaluation of GPC activity in controlled clinical studies in cerebrovascular patients with cognitive dysfunction.

### ALLOGRAFT TRANSPLANTATION OF GANGLIONIC EMINENCE EMBRYONIC STEM CELLS IN A RAT MODEL OF HUNTINGTON'S DISEASE

M. Tropiano, M. Boido, A. Vercelli

Neuroscience Institute Cavalieri Ottolenghi (NICO), Dept. of Neuroscience, University of Turin, Regione Gonzole 10, Orbassano (Italy)

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by the dominant mutation of the huntingtin gene on chromosome 4, first described by G. Huntington in 1872. The mutation results in a progressive atrophy of the basal ganglia. The neurodegenerative process is very selective, affecting the only GABAergic medium-sized spiny neurons, that project from the

striatum to the globus pallidus and nucleus subthalamicus. In order to reproduce the striatal neuropathological features of HD, quinolinic acid (QA) represents the most commonly used glutamate agonist in both rodent and primate models of HD, leading to the selective loss of striatal GABAergic neurons. Since it is unable to cross the bloodbrain barrier, QA needs to be directly injected into the striatum.

Here, we have performed a single unilateral intrastriatal infusion of 240 nmol QA in Sprague-Dawley male rats (weighing 250-300g). This induced an evident rotational behavior of the animal, immediately after injury. Fifteen days after the injection, the animals were sacrificed and the brains dissected. By Nissl staining, we analyzed the brain parenchyma and measured the ipsilateral ventricle volume by NeuroLucida software: its size was markedly increased (about 23%) compared to the contralateral one. Additionally we observed a massive astrogliosis (highlighted by GFAP-immunohistochemistry - IHC) and a strong microglial activation (IBA1 IHC). To assess potential restorative therapies, one week after QA injection, a separate group of injured rats received 100,000 EGFP-positive embryonic (E14) neural cells obtained from the murine medial ganglionic eminence. To reduce the immune response induced by graft, animals received an oral treatment with 10 mg/kg cyclosporine A, the day before transplantation and the following 2 days.

After 15 days from transplantation, we found a great number of injected survived cells, in absence of signs of reactions of the host. Grafted cells were differentiated in both glial and neuronal cells, which extended their processes in the host tissue, thus appearing well integrated into the host tissue. Although these are preliminary observations, we are currently extending our studies to improve the lesion model, to better evaluate transplanted cell survival and differentiation, their role within striatum and the possibility to create connections with other host neuronal populations.

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## THEME 4 NEUROPLASTICITY

### CONDITIONED MEDIUM OF OLFACTORY ENSHEATHING OR NEUROBLASTOMA B104 CELLS PROMOTES DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE TOWARD A NEURAL PHENOTYPE

R. Pellitteri<sup>1</sup>, D. Lo Furno<sup>2</sup>, A. Graziano<sup>2</sup>, R. Giuffrida<sup>2</sup>, C. Venera<sup>2</sup>

<sup>1</sup>Institute of Neurological Sciences, National Research Council, Section of Catania; <sup>2</sup>Dept of Bio-medical Sciences, Section Physiology, University of Catania, Italy

The mammalian olfactory system is one of the few areas of the central nervous system able of continuous neurogenesis throughout lifetime supported by peculiar glial cells of the olfactory nerve, called Olfactory Ensheathing Cells (OECs). They share phenotypic characteristics with Schwann cells (SCs) and astrocytes and represent a source of several trophic factors. On the other hand, B104 neuroblastoma cells are recognized to induce differentiation of neural stem cells into oligodendrocyte precursor cells. Considering as described, in this work, we studied the effect of both B104 and OECs conditioned medium on cultured adipose tissue-derived mesenchymal stem cells (AT-MSCs) and verified if these conditioned media were capable of inducing them to a neuronal phenotype. In order to this goal, AT-MSCs cultures were obtained from 10 donors undergoing abdominal liposuction procedures. OECs were isolated from 2-day old rat pup (P2) olfactory bulbs, the medium was collected after 24-48 h and stored (-20°C) until further use. The B104 neuroblastoma cell line was maintained in DMEM/FBS, after 3 days the medium was collected, filtered and stored at -80°C. After reaching confluence, AT-MSCs were trypsinized and subcultured in multi-well plates for 24 h. The medium was removed and replaced with OECs-CM and/or B104-CM. Some wells were used as control and grown only with DMEM/FBS. Some samples were used for immunocytochemistry and others for flow cytometry. Some neural markers, such as nestin, PGP 9.5 and MAP2 in a time-dependent manner. They display morphological features resembling neuronal cells, and result negative for GFAP and A2B5, astrocyte and oligodendrocyte markers, respectively. This study demonstrates that AT-MSCs could be influenced by the environment toward a neuronal phenotype. This culture system may offer many advantages as potential material for replacement therapy in central nervous system degenerative diseases.

### CYTO-ARCHITECTURE OF PRE-LIMBIC CORTEX IN AN ANIMAL MODEL OF SCHIZOPHRENIA

G. Casu<sup>1,2</sup>, M.A. Casu<sup>1</sup>, S. Marcello<sup>1,2</sup>, A. Pilleri<sup>1,2</sup>, G. Manca<sup>1,2</sup>, E. Musu<sup>3</sup>, G. Marchese<sup>1</sup>

<sup>1</sup>Institute of Translational Pharmacology, UOS of Cagliari, National Research Council; <sup>2</sup>Neuroscienze-Pharmaness Scarl; <sup>3</sup>Telemicroscopy Lab, Sardegna Ricerche/CRS4 - Scientific and Technological Park of Sardinia POLARIS, Loc. Piscinamanna, Pula, Cagliari, Italy

The rat neonatal ventral hippocampal lesion (NVHL) is a reliable neurodevelopmental animal model of schizophrenia, in which an incorrect maturation of medial pre-frontal cortex may

possibly account for the appearance of rat behavioral alterations after puberty. In the present study, morphometric analyses have been carried out in order to ascertain the presence of possible morphological alterations in the pre-limbic cortex of NVHL rats. Fractionator stereological analyses indicated that pre-limbic cortex volume is reduced in this animal model, without a significant reduction of the total number of NeuN-labeled neurons. NVHL also affected dendrite and spine density of Golgi-Cox stained pyramidal neurons in this brain area. Furthermore, ultra-microscopy analyses highlighted that the density of asymmetric synapses is reduced in the pre-limbic cortex of lesioned rats when compared with sham rats. The results of this study are in line with clinical brain imaging data indicating that a shrinkage of frontal cortex may occur in schizophrenia. Furthermore, the reduced density of asymmetric synapses is in agreement with a possible impairment of pre-frontal cortex excitatory input in schizophrenia. FIB-SEM 3D-reconstructions are in progress to evaluate possible alterations of synaptic contacts and volumes.

### DIFFERENTIAL EFFECTS OF ACUTE COCAINE ON ERK PHOSPHORYLATION IN THE LIMBIC SYSTEM OF PSYCHOGENETICALLY SELECTED ROMAN HIGH- AND LOW-AVOIDANCE RATS

M.A. Piludu<sup>1</sup>, M. Rosasa<sup>1</sup>, I. Sabariego<sup>1</sup>, V. Giugliano<sup>1,2,3</sup>, O. Giorgi<sup>1</sup>, E. Acquas<sup>1,2,3</sup>

<sup>1</sup>Dept. of Life and Environmental Sciences, University of Cagliari; <sup>2</sup>Centre of Excellence for the Neurobiology of Addiction, University of Cagliari; <sup>3</sup>CNR Institute of Neuroscience, Cagliari, Italy

The Roman low- (RLA) and high- (RHA) avoidance rats are selectively bred for, respectively, poor vs. rapid acquisition of active avoidance in a shuttle box and differ markedly in the propensity to self-administer cocaine. Thus, compared with their RLA counterparts, RHA rats learn to self-administer cocaine faster and consume larger amounts of the drug. Acute and chronic cocaine administration induce plastic adaptations of cellular mechanisms underlying learning and memory in the limbic system of the rat brain, and such plastic changes involve signaling by extracellular signal-regulated kinase (ERK). The present study was therefore designed to compare the effects of the acute administration of cocaine on ERK phosphorylation in limbic areas of RHA and RLA rats, including the infralimbic prefrontal cortex (PFCx-IL), prelimbic PFCx (PFCx-PrL), shell and core compartments of the nucleus accumbens (Acb-sh and Acb-co, respectively), amygdala, and bed nucleus of the stria terminalis (BNST). Rats were sacrificed 20 min after the administration of cocaine (5 mg/kg, i.p.) and the number of p-ERK positive neurons was determined by light microscopy in the regions of interest of immunohistochemically stained brain slices. Acute cocaine induced a larger increase in the number of p-ERK positive neurons in the Acb-sh and in the PFCx-IL of RHA vs. RLA rats, but failed to modify p-ERK immunoreactivity in the Acb-co and the PFCx-PrL of either line. Moreover, no line- or cocaine-related changes were observed in the amygdala and BNST. Cocaine increased ERK phosphorylation in the PFCx-IL and the Acb-sh, two brain areas belonging to the limbic subcircuit which controls motivated behaviors that play a crucial role in the initial response to the drug, but had no effect on the PFCx-PrL and Acb-co, two areas that are part of a motor subcircuit involved in habit acquisition upon long term exposure to cocaine. Furthermore, the increment in the number of p-ERK positive neurons was observed exclusively in RHA rats, which are more susceptible to develop cocaine self administration than RLA rats. These results support the view that the Roman lines represent a valid model to investigate the neural underpinnings of the individual vulnerability to drug addiction.



## STRUCTURAL SYNAPTIC REARRANGEMENTS IN ACCUMBAL SHELL SPINY NEURONS DURING ETHANOL WITHDRAWAL

F. Piras<sup>1</sup>, G. Mulas<sup>1</sup>, M. Diana<sup>2</sup>, S. Spiga<sup>1</sup>

<sup>1</sup>Dept. of Life and Environmental Sciences, University of Cagliari, Cagliari; <sup>2</sup>"G.Minardi" Laboratory of Cognitive Neuroscience, University of Sassari, Sassari, Italy

The nucleus accumbens (Nacc) plays a significant role in reward circuit and its activity is heavily modulated by glutamate (GLU) and dopamine (DA) containing projections that originate from cortical and limbic regions respectively. These projections converge on a common postsynaptic target: the Medium Spiny Neuron (MSN) especially in the shell of n. accumbens. While the cortical and hippocampal glutamate afferents make asymmetric synapses on MSN's dendritic spines head, the dopaminergic afferents, arising from VTA, make symmetric synapses with spine's neck, creating the so called "striatal microcircuit" or "synaptic triad". Many drugs of abuse, like ethanol, interfere with the DAergic pathway, causing neuronal aberrations and alterations on their connectivity. In this study, ethanol dependence was induced by the chronic exposure of ethanol-containing liquid, to obtain chronically and abstinent (EtOH-W) rat groups. The simultaneous visualization of the TH positive terminals, the PSD-95 positive antigens and Golgi-impregnated MSNs in the shell, allowed us to study, the qualitative and quantitative relationships between these elements. In particular, in the shell, TH-positive fibers density was decreased by about 50%, accompanied by a similar reduction in PSD-95 immunoreactivity selectively in EtOH-W vs control rats group. Contrary no statistical changes were observed in chronically treated rats. Counts showed contemporary loss of long thin spines density selectively in the MSN of the EtOH-W rats. Interestingly no statistical differences were found in other category of spines. The selective loss observed here may offer further hints on the neurobiological underpinnings of cognitive dysfunction of alcohol abuse and alcoholism.

## SESSION II EXPERIMENTAL PARKINSON'S DISEASE

### DROSOPHILA MODEL FOR PARKINSON'S DISEASE: FUNCTION, ANATOMY AND SCREENING WITH PHYTOTERAPICS FROM SARDINIAN AND INDIAN FLORA (WITHANIA SOMNIFERA AND MUCUNA PRURIENS)

S. Poddighe<sup>1</sup>, F. De Rose<sup>2</sup>, M.D. Setzu<sup>1</sup>, P. Solla<sup>3</sup>, R. Marotta<sup>4</sup>, R. Ruffilli<sup>4</sup>, E. Acquas<sup>2</sup>, S. Kasture<sup>5</sup>, F. Marrosu<sup>3</sup>, A. Liscia<sup>2</sup>

<sup>1</sup>Dept. of Biomedical Sciences, University of Cagliari, Cagliari; <sup>2</sup>Dept. of Life and Environmental Sciences, University of Cagliari, Cagliari; <sup>3</sup>Dept. of Public Health, Clinical and Molecular Medicine, University of Cagliari, Cagliari; <sup>4</sup>Nanobiotech Facility, Italian Institute of Technology, Genova, Italy; <sup>5</sup>Sanjivani College of Pharmaceutical Education & Research, Ahmednagar, Kopergaon, India

Parkinson's disease (PD) is one of the most common neurodegenerative diseases characterized by the clinical triad: tremor, akinesia and rigidity as well as olfactory disturbances. The fruit fly *Drosophila melanogaster* is becoming a powerful model organism to study neurodegenerative diseases. *PINK1* mutants display many important diagnostic symptoms of the disease such as akinetic motor behavior. We sought to use this system to explore, besides the motor, also the olfactory dysfunction, if any, in *PINK1* mutants. In the present study, we describe for the first time, neurophysiological and neuroanatomical results concerning the olfactory function in *PINK1* mutant flies. In this respect, electrophysiological responses to synthetic and natural volatiles (essential oils) were recorded from groups of *PINK1* mutant adults at three different time points in their life cycle. In addition, the olfactory and the motor behavior (climbing activity) were recorded in the same age matched groups. We found that mutant adults showed a decrease in the olfactory response to several chemicals and essential oil volatiles. The results showed that the olfactory response as well as the locomotor behavior in mutant adults decreased even more as the flies aged. Immunohistological analysis of the antennal lobes (ALs) in these mutants revealed structural abnormalities, especially in the expression of Bruchpilot protein, a marker for synaptic active zones. Transmission electron microscopy on presynaptic active zones pointed out mitochondrial alteration in cholinergic neurons projecting to the ALs. The combination of electrophysiological and morphological results suggests that the altered synaptic organization may be due to a neurodegenerative process. The phytochemicals delivery by feeding *PINK1* mutants with *M. pruriens* and *W. somnifera* had different effects in increasing lifespan and in enhancing both the defective olfactory and locomotor behavior.

Our results indicate that this model can be used as a tool for understanding PD pathogenesis and pathophysiology. These data help to explore the potential of using *drosophila* as a model for monitoring PD progression and developing new treatments.

### **SELECTIVE CHANGES IN DENDRITIC SPINES OF STRIATAL MEDIUM SPINY NEURONS IN A CHRONIC MOUSE MODEL OF PARKINSON'S DISEASE**

C. Mura<sup>1</sup>, G. Mulas<sup>1</sup>, A. Piga<sup>1</sup>, R. Podda<sup>1</sup>, A.R. Carta<sup>2</sup>, D. Lecca<sup>2</sup>, S. Spiga<sup>1</sup>

<sup>1</sup>Dept. of Life and Environmental Sciences, University of Cagliari; <sup>2</sup>Dept. of Biomedical Sciences, University Cagliari; Italy

Parkinson's disease (PD) is characterized by a progressive degeneration of mesencephalic dopamine (DA) containing neurons and gradual worsening of motor functions. A series of cellular and synaptic alterations occurs in striatal medium spiny neurons (MSN) in response to the massive dopaminergic loss. In particular, dysfunctional DAergic and glutamatergic NMDA transmission on MSN may contribute to the clinical features of PD.

In this study we investigated neurodegeneration and morphological changes in MSN in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid (MPTP) chronic mouse model of PD. MPTP (25 mg/kg) plus probenecid (100 mg/kg) were administered twice a week for 5 weeks.

Using Golgi staining combined with immunofluorescence and confocal microscopy, we assessed tyrosine hydroxylase (TH)-immunoreactivity and morphological details in impregnated striatal MSN. TH-immunoreactivity was evaluated in the substantia nigra pars compacta (SNc) to quantify the effective loss of DAergic neurons, in the dorsal striatum to quantify TH-positive fibers and in the nucleus accumbens shell taken as a control. Furthermore, dendritic spine density of MSN was measured in the dorsal striatum and accumbens shell.

As expected, TH positive cells were decreased in number in the SNc, and TH terminals were consequently reduced in the dorsal striatum. Interestingly no changes in TH-immunoreactivity was observed in the shell. Moreover, dendritic long-thin spines were selectively reduced in density in MSN of the dorsal striatum as compared to control mice. In contrast, no changes were found in MSN in the shell. In summary, results show that nigro-striatal degeneration was associated with selective changes in dendritic spines and synaptic connectivity in MSNs of the dorsal striatum, which may contribute to the development of motor impairment.

### **EFFECT OF THE SEROTONIN PRECURSOR, 5-HYDROXYTRYPTOPHAN, ON L-DOPA-INDUCED DYSKINESIA IN PARKINSONIAN RATS**

E. Tronci, C. Lisci, R. Stancampiano, F. Camino, P. Devoto, M. Collu, M. Carta

Dept. of Biomedical Sciences, University of Cagliari, Italy

The serotonin system has recently emerged as an important player in the appearance of L-DOPA-induced dyskinesia (LID) in experimental models of Parkinson's disease, as it provides an unregulated source of L-DOPA-derived dopamine release in the dopamine-depleted striatum. Accordingly, toxin lesion or pharmacological silencing of the serotonin neuron suppressed LID in the rat and monkey models of Parkinson's disease. However, 5-HT<sub>1</sub> receptor agonists were also found to partially reduce the therapeutic effect of L-DOPA. In this study, we evaluated whether an increase of the serotonin tone induced by the administration of the serotonin precursor 5-hydroxytryptophan (5-HTP) could affect induction and expression of LID, as well as the therapeutic effect of L-DOPA, in 6-OHDA-lesioned rats.

Drug naïve and L-DOPA-primed 6-OHDA-lesioned rats were chronically treated with a daily injection of L-DOPA (6 mg/kg

plus benserazide, s.c.) alone, or in combination with 5-HTP (24-48 mg/kg, i.p.). The abnormal involuntary movements (AIMs) test, as well as the stepping and the motor activity tests, were performed during the chronic treatments.

Results showed that 5-HTP reduced the appearance of LID of about 50% at both tested doses. A partial reduction of the therapeutic effect of L-DOPA was seen with the higher but not with the lower dose of 5-HTP. 5-HTP 24 mg/kg was also able to reduce the expression of dyskinesia in L-DOPA-primed dyskinetic rats, to a similar extent than in L-DOPA-primed rats.

Importantly, the antidyskinetic effect of 5-HTP 24 mg/kg does not appear to be due to a competition with L-DOPA for crossing the blood brain barrier; in fact, similar L-DOPA levels were found in L-DOPA only and L-DOPA plus 5-HTP 24 mg/kg treated animals. These data suggest that 5-HTP may be useful to counteract the appearance of dyskinesia in Parkinson's disease patients.

### **INVESTIGATION OF THE ANTIDYSKINETIC EFFECT OF MEMANTINE IN THE RAT PARKINSON'S DISEASE MODEL**

C. Fidalgo, E. Tronci, C. Lisci, M. Collu, R. Stancampiano, M. Carta

Dept. of Biomedical Sciences, Cagliari University, Cagliari, Italy

Although L-DOPA remains the most effective drug to treat motor symptoms in Parkinson's disease (PD), its long-term administration leads to development of dyskinesia. An increasing body of experimental evidence demonstrates that the glutamate system is involved in the appearance of dyskinesia. In fact, the NMDA receptor antagonist amantadine is the only antidyskinetic compound used in patients, albeit with limited efficacy and side effects. Here, we studied the effect of memantine, another NMDA receptor antagonist, in clinical use for the treatment of dementia, in the rat model of L-DOPA-induced dyskinesia. 6-OHDA-lesioned rats were made dyskinetic by a chronic L-DOPA treatment, and then subjected to pharmacological challenges with memantine (5, 10, 15 and 20 mg/kg) in combination with L-DOPA (6 mg/kg). Abnormal involuntary movements (AIMs) were evaluated according to the scale developed by Lee et al. (1999). Results showed that acute treatment with memantine at 15 and 20 mg/kg produced significative reduction of AIMs in already dyskinetic rats, suggesting that memantine has, indeed, antidyskinetic properties, as previously shown for amantadine in the same model. In a second set of experiments, 6-OHDA-lesioned drug-naïve rats were subchronically treated with L-DOPA (6 mg/kg plus benserazide) alone or in combination with memantine 20 mg/kg in order to study whether administration of memantine from the first dose of L-DOPA could prevent development of dyskinesia. However, when given subchronically in drug-naïve animals, the effect of memantine against LID was not maintained over time, suggesting that desensitization of the NMDA receptors is rapidly induced following repeated exposure to this drug. Interestingly, a 3-week washout period did not appear to reverse this desensitization. Our results are in line with clinical observations suggesting that NMDA antagonism is only transiently effective against L-DOPA-induced dyskinesia in Parkinson's disease patients.

## SESSION III - SENSORY SYSTEMS

### MORPHO-FUNCTIONAL CHARACTERIZATION OF TASTE NEURONS IN LABELLAR APPARATUS IN THE MEDFLY

#### *Ceratitis capitata* (Wied.)

C. Masala<sup>1</sup>, F. Loy<sup>2</sup>, P. Sola<sup>1</sup>, G. Sollai<sup>1</sup>, P. Muro<sup>1</sup>, R. Crnjar<sup>1</sup>

<sup>1</sup>Dept. of Biomedical Sciences, Section of Physiology, University of Cagliari, Monserrato (CA); <sup>2</sup>Dept. of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Monserrato (CA), Italy.

The medfly (*Ceratitis capitata* Wied.) is a widespread pest for horticulture, mainly targeting fruits of pomaceous and citrus cultivars. Scanty data is available about its chemosensory responses to contact salty, sweet, sour and bitter stimuli. Aim of the present work is to provide a morphofunctional map of the taste labellar apparatus, by investigating the relative distribution of different sensillum types and evaluating spike activity recorded from the labellar sensilla in response to NaCl, fructose, acids (citric and malic) and bitter compounds (quinine, quercetin and PROP). HRSEM and TEM observations indicate the presence of 6 pairs of Long type and about 40 pairs of Intermediate type labellar chemosensilla, each containing 4 different neurons. By analysis of spike waveforms in the responses, 4 neurons were identified responding to different stimuli in both sensillum types of *C. capitata*. The response specificity of three of these cells (named M1, M2 and S) was determined on the basis of their dose-response profiles. M1 was specific to NaCl, M2 to fructose. Both acids appeared to excite the S cell and to inhibit the M1 cell. Citric acid also partly inhibited the response of M2 cell. No cell clearly responded to any of the bitter stimuli. None of them elicited any response from each of the four neurons in both Long and Intermediate type sensilla. In the light of our data, we assume that labellar sensilla do not respond to deterrent stimuli. This fact is not so unusual as previous studies in *Drosophila* revealed that Long type sensilla respond to salt and sweet but not to bitter stimuli, while Intermediate and Short type sensilla are activated by only a few bitter substances.

The comprehension of the medfly taste responses may be critical for population control program.

### MORPHO-FUNCTIONAL ANALYSIS OF INDIVIDUAL DIFFERENCES OF PROP BITTER TASTE PERCEPTION

M. Melis<sup>1</sup>, P. Muro<sup>1</sup>, T. Cabras<sup>2</sup>, I. Messana<sup>2</sup>, R. Crnjar<sup>1</sup>, I. Tomassini Barbarossa<sup>1</sup>

<sup>1</sup>Dept. of Biomedical Sciences, University of Cagliari; <sup>2</sup>Dept. of Life and Environmental Sciences, University of Cagliari, Italy

Taste sensitivity varies greatly among individuals. A well-known example of this variability is the genetic ability to taste bitter thiourea compounds, such as 6-n-propylthiouracil (PROP). Individuals are defined tasters (super-tasters and medium tasters) or non-tasters based on their ability to discriminate this compound. PROP sensitivity is associated with the bitter receptor gene *TAS2R38* haplotypes, and with differences in the density of fungiform papillae. Although most of PROP phenotypic variations are explained by the allelic diversity of the bitter receptor *TAS2R38*, they cannot explain the PROP taster status-related differences in the perception to different oral stimuli. In order to identify and characterize other factors that may be involved in the expression of this human trait, 63 subjects were genotyped for *TAS2R38* and the salivary trophic factor (gustin) gene by PCR techniques. Besides, their PROP sensitivity was assessed by

threshold and supra-threshold methods and fungiform papilla density, diameter and morphology were determined. Moreover, the quantitative and qualitative determination of salivary proteins was performed by HPLC-ESI-MS analysis in super-tasters and non-tasters individuals before and after PROP stimulation. Gustin and *TAS2R38* genotypes were associated with PROP threshold, while bitterness intensity was mostly determined by *TAS2R38* genotypes. Fungiform papillae densities were associated with both genotypes (with a stronger effect for gustin), but papilla morphology was a function of gustin alone. In addition, basal levels of II-2 and Ps-1 proteins, belonging to the basic proline-rich protein (bPRPs) family, were significantly higher in PROP super-taster than in non-taster unstimulated saliva, and PROP stimulation elicited a rapid increase in the levels of these same proteins only in PROP super-taster saliva. In conclusion, among the factors contributing to individual differences of PROP sensitivity, in addition to the *TAS2R38* variants with its different affinity for the stimulus, we found: 1) a gustin gene polymorphism that, by modulating the protein activity, controls the growth and maintenance of taste buds; 2) the specific salivary proteins of bPRP family (Ps-1) that could be involved in twist and turn of the PROP molecule, thus facilitating its binding with the receptor.

### LOCUS K: A NOVEL TERRITORY OF THE HUMAN DORSAL COLUMN NUCLEI

M.P. Serra, M. Quartu, M. Boi, L. Poddighe, C. Picci, T. Melis, M. Del Fiocco

Dept. of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Monserrato, Italy

In the past years, this research group has reported the occurrence, in the human newborn and adult medulla oblongata, of discrete gray matter subregions, located within the boundaries of the cuneate nucleus and fascicle, that hold neurochemical features in common with second order sensory nuclei for protopathic neurotransmission (Del Fiocco et al., Brain Struct. Funct. 2013, DOI 10.1007/s00429-013-0625-4 and references therein). Those areas appear distinct from the known subdivisions of the dorsal column nuclear complex described by early and recent literature. Here, we provide a histoarchitectural characterization of those subregions and propose a denomination for them. The cyto- and myeloarchitectural features of gray matter areas of the cuneate territory, identified by their resemblance to the caudal part of the spinal trigeminal sensory nucleus (Sp5C) in the immunoreactivity to a number of neurochemical markers, are examined in the human newborn and adult medulla oblongata. Computerized analysis of cell size and density shows that, in adult tissue, the mean cell diameter of Nissl stained neurons ranges 6-32 (mean 15)  $\mu\text{m}$  in the main cuneate nucleus, and 5-18 (mean 9)  $\mu\text{m}$  in both the neurochemically-identified cuneate subregions and Sp5C substantia gelatinosa. In the same three regions, the mean cell density values amount to 159/mm<sup>2</sup>, 872/mm<sup>2</sup>, and 560/mm<sup>2</sup>, respectively. Differences in cell size and density among the three regions are statistically significant. Luxol fast and Black Gold kit II staining shows that myelinated fibres, abundant in the main cuneate nucleus, are scarce in both the discrete cuneate subregions and Sp5C substantia gelatinosa. Functional, preclinical and clinical studies show that the dorsal column nuclei, classical relay station of fine somatic tactile and proprioceptive sensory stimuli, are also involved in pain neurotransmission. Our data support the concept that the identified cuneate subregions represent a special component of the human dorsal column nuclei that shares neurochemical and cyto- and myeloarchitectural features with sensory nuclei that relay protopathic stimuli, including pain. After the ancient name of our town, we propose to name it Locus Karalis and, briefly, Locus K.

## SESSION IV NEUROPATHOLOGICAL STUDIES – HUMAN TISSUE AND ANIMAL MODELS

### NEUROPATHOLOGICAL ANALYSIS AND CLINICAL CORRELATES OF CHRONIC CONSTIPATION IN PATIENTS WITH PARKINSON'S DISEASE

F. Giancola<sup>1,2</sup>, C. Sorteni<sup>1</sup>, F. Torresan<sup>2</sup>, M. Guarino<sup>3</sup>, G. Barbara<sup>2</sup>, C. Cremon<sup>2</sup>, E. Boschetti<sup>2</sup>, R. Latorre<sup>2</sup>, R. Chiochetti<sup>1</sup>, C. Vallorani<sup>1</sup>, P. Clavenzani<sup>1</sup>, P. Cortelli<sup>4</sup>, V. Stanghellini<sup>2</sup>, C. Sternini<sup>5</sup>, R. De Giorgio<sup>2</sup>

<sup>1</sup>Dept. of Veterinary Medical Science, University of Bologna, Bologna; <sup>2</sup>Dept. of Medical and Surgical Sciences / Digestive Diseases and Internal Medicine, University of Bologna and St.Orsola-Malpighi Hospital, Bologna; <sup>3</sup>Neurology Unit, Dept. of Internal Medicine, Aging and Nephrology, Sant'Orsola-Malpighi Hospital, Bologna; <sup>4</sup>Dept. of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy; <sup>5</sup>CURE/Digestive Diseases Research Center, Division of Digestive Diseases, Dept. of Medicine and Neurobiology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California, USA

Chronic constipation (CC) represents one of the most common gastrointestinal (GI) complaint in Parkinson's disease (PD), being diagnosed in about 80% of patients. Furthermore, CC is one of the earlier manifestations of PD, often preceding the somatic motor impairment. The enteric nervous system (ENS), controlling gut functions, can be a target of the PD-related degenerative processes as Lewy bodies and neurites can be detected in myenteric and submucosal neurons of PD tissue specimens. However, the precise neurochemical ENS abnormalities underlying CC/PD patients remain largely unknown. Our aims in CC/PD patients were to: 1) characterize constipation by assessing colonic transit time (TT) and anorectal manometry (AM); 2) analyze colonic submucosal neurons of PD patients vs controls, particularly assessing the secretomotor neuron component. GI symptoms were evaluated by the Rome III questionnaire, while PD was established by a Unified Parkinson's Disease Rating Scale (part III). CC was completely studied in 16 PD patients (7F, 9M; age range: 64-85 yrs) by TT and AM and colonoscopy; 10 control subjects (3F, 7M; age range: 33-77 yrs) undergoing screening colonoscopy were also enrolled in the study. Using routine biopsies during colonoscopy, we obtained submucosal specimens with related neural network in 10 CC/PD patients and 10 controls. The submucosal plexus was studied by immunohistochemistry on whole mount preparations using a mouse monoclonal anti-HuC/D as pan-neuronal marker (Invitrogen, 1:50) and a rabbit polyclonal anti-VIP (vasoactive intestinal peptide-7913; CURE/DDRC, UCLA, 1:2500) antibodies. Four groups of CC/PD patients were characterized: a) 47% showed a delayed TT and altered AM; b) 20% had only a delayed TT; c) 20% only an altered AM; d) the remaining 13% had no evident functional impairment. There were no significant differences in the number of HuC/D immunoreactive (-IR) neurons/ganglion between CC/PD ( $3.6 \pm 1.2$ ) and controls ( $3.8 \pm 1.9$ ); however, a reduced number of VIP-IR neurons was found in CC/PD ( $74.4 \pm 19.9$ ) vs controls ( $91.0 \pm 11.5$ ).

Most (87%) of CC/PD patients has a marked impairment of colonic motor and rectal sensory functions. Neurochemical changes in a subset of secretomotor neurons suggest that altered secretory mechanisms may accompany sensorymotor dysfunction in PD-related CC pathophysiology.

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### STRESS GRANULE ASSEMBLY IN CULTURED FIBROBLAST FROM TDP-43 MUTANT ALS PATIENTS

P. Coni<sup>1</sup>, C. Brancia<sup>1</sup>, A. Floris<sup>2</sup>, I. Bellini<sup>2</sup>, S. Orrù<sup>2</sup>, V. Sogos<sup>1</sup>

<sup>1</sup>Dept. of Biomedical Sciences, University of Cagliari; <sup>2</sup>Medical Genetics, Dept. of Medical Sciences, University of Cagliari, Italy

Stress granules (SGs) are cytoplasmic structures composed of non-translating messenger ribonucleoproteins (mRNPs) that rapidly aggregate in cells exposed to adverse environmental conditions. SGs function in part to triage RNA and sequester transcripts not needed for coping with the stress. Several neurodegenerative diseases are associated with the accumulation of abnormal proteins, which serve as histologic hallmarks of specific disorders. The RNA-binding protein TDP-43 is strongly linked to Amyotrophic Lateral Sclerosis (ALS). In fact, mutations in the gene encoding TDP-43 are associated with ALS. Moreover, this protein is also a major constituent of pathological intracellular inclusions in this disease.

We investigated the dynamic of SGs assembly in human cultured fibroblasts from ALS patients carrying TARDBP<sup>A382T</sup> mutation compared with fibroblast from healthy subjects. In order to induce SGs formation, cells were treated with sodium arsenite (SA) at different concentrations (0.5 mM, 1 mM) and exposure times (30 min, 1h). SGs were identified by immunostaining for TIA-1 (T-cell internal antigen-1), an early marker for SGs and Hur-1 (Human antigen R). After 30 minutes, we observed small and sporadic cytoplasmic inclusions TIA-1 positive in both groups of cells. After 1h the TIA-1 immunostained granules were bright and numerous. Interestingly, the number of cells exhibiting SGs were significantly higher in fibroblast from ALS patients compared to healthy controls. On the contrary, TDP-43 immunostaining was only observed into the nucleus of all the cells.

These data suggest that TDP-43 may modulate stress granule formation contributing to the cellular response to acute stress. Thus, we may hypothesize that an altered control of mRNA translation in stressful conditions may induce motor neuron degeneration.

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### BRAIN AND CERVICAL LYMPH NODE DENDRITIC CELLS DURING CENTRAL NERVOUS SYSTEM INFECTION

C. Laperchia<sup>1</sup>, G. Grassi-Zucconi<sup>1</sup>, A. Andrioli<sup>1</sup>, N. Van Reet<sup>2</sup>, F. Florenzano<sup>3</sup>, P. Büscher<sup>2</sup>, M. Buffelli<sup>1</sup>, M. Bentivoglio<sup>1</sup>

<sup>1</sup>Dept. of Neurological and Movement Sciences, University of Verona, Verona, Italy; <sup>2</sup>Institute of Tropical Medicine, Antwerp, Belgium; <sup>3</sup>European Brain Research Institute (EBRI), Rome, Italy

Dendritic cells (DCs) are a subset of leukocytes which promote the immune response by antigen presentation to T cells. In the central nervous system (CNS), DCs are poorly explored during inflammation and infection. Both DCs and brain-derived antigens are drained by cerebrospinal fluid in the afferent lymphatic vessels of cervical lymph nodes, where antigen presentation mainly occurs. We recently demonstrated that in the *thy1GFP-M* transgenic mice DCs express green fluorescent protein (GFP). These mice represent, therefore, a novel animal model for the study of DCs *in vivo* by two-photon microscopy (TPM). Here, we investigated DCs in an experimental model of CNS infection represented by African trypanosomiasis, also

known as sleeping sickness. The causative agent of this disease, which is deadly if untreated, is the parasite *Trypanosoma brucei* (*Tb*). After infection, the disease evolves from a first, hemolymphatic stage, to a second, meningoencephalitic stage, in which both T cells and parasites cross the blood-brain barrier and enter the brain parenchyma by mechanisms that still require full clarification. Our aim was to understand whether DCs lead upstream events for T cells/*Tb* neuroinvasion. *In vivo* transcranial acquisition by TPM in *thy1GFP-M* mice infected with fluorescent trypanosomes (Ds-Red *Tb*) showed, during the hemolymphatic stage, direct interactions in the blood vessels between DCs and Ds-Red *Tb*, possibly representing antigen capture events. Moreover, during the early meningoencephalitic stage, we found that DCs are recruited from the blood and invade the brain parenchyma, where they exhibit random motions. At an advanced phase of the disease, DCs are mainly arranged in static clusters which incorporate the parasite. Confocal analysis showed that the sub-capsular zone of cervical lymph nodes, where DCs are rare or absent in basal conditions, is invaded by migratory DCs during the late meningoencephalitic stage; at the same time point, both migratory and resident DCs preferentially contact CD8+ T cells, probably to present *Tb* antigens and to prime the immune-response. The present findings provide for the first time evidence of a dynamic role of DCs during CNS infection and point to a role of DCs in mechanisms of pathogen neuroinvasion.

#### ROLE OF NG2 ON STRUCTURAL MODULATION OF BBB-ENDOTHELIAL TIGHT JUNCTIONS DURING DEVELOPMENT AND IN A MURINE MODEL OF EAE

M. Errede<sup>1</sup>, G. Ferrara<sup>2</sup>, M. Rizzi<sup>1</sup>, F. Girolamo<sup>1</sup>, L. Roncali<sup>1</sup>, D. Virgintino<sup>1</sup>

<sup>1</sup>Dept. of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari School of Medicine, Bari; <sup>2</sup>Dept. of Neurosciences Ophthalmology, Genetics, Rehabilitation and Child Health, University of Genoa, Italy

Cell components that participate in neurovascular unit (pericytes, astrocytes, oligodendrocytes, microglia, neurons) may influence the blood-brain barrier (BBB) phenotype of endothelial cells through mutual cell-associated molecule interactions. Immature and activated brain pericytes, which are known to play a critical role in blood vessel stability and BBB differentiation, express high levels of the transmembrane chondroitin sulfate proteoglycan NG2, which is also widely expressed in progenitor cells, where it promotes cell development, proliferation and migration. This study aimed to investigate the possible role of NG2 proteoglycan on BBB-endothelial cells of brain and spinal cord microvessels, through the analysis of tight junction (TJ) proteins in normal and experimental conditions. The chosen pathological model was the MOG-induced experimental autoimmune encephalomyelitis (EAE), an experimental model of human multiple sclerosis characterized by BBB dysfunction, including endothelial TJ alterations. Four groups of mice were analysed: (1) wild type (WT), (2) homozygous NG2 knock out (NG2KO), (3) WT affected by EAE (WT-EAE), and (4) NG2KO affected by EAE (NG2KO-EAE). Expression and distribution of the TJ transmembrane proteins, claudin-5 and occludin, and the level of microglia/macrophage inflammatory infiltrates, were analyzed by immunohistochemistry and laser confocal microscopy. BBB function of cortex and spinal cord microvessels was also estimated by FITC-dextran experiments. Compared to the typical TJ staining pattern observed in brain and spinal cord microvessels of WT mice, NG2KO vessels showed thinner junctional strands with alternated TJ protein aggregates. TJs of WT-EAE mice were characterized by severe

TJ alterations consisting in an interrupted TJ protein staining pattern and leakage of the tracer into the perivascular neuropil together with Iba+ infiltrates. On the contrary, in NG2KO-EAE mice, claudin-5 and occludin formed an apparently unaffected linear and continuous staining, which correspond to a reduced presence of Iba-1 positive cells and a moderate barrier leakage. These results demonstrate the involvement of NG2 proteoglycan on TJ protein arrangement in normal microvessels and suggest a possible compensatory/protective role of NG2 ablation in pathological conditions characterized by CNS inflammatory response.

#### ISOFORMS OF NG2/CSPG4 PROTEOGLYCAN ARE EXPRESSED IN HUMAN FETAL BRAIN AND GLIOBLASTOMA

D. Virgintino<sup>1</sup>, F. Girolamo<sup>1</sup>, M. Rizzi<sup>1</sup>, A. Dallatomasina<sup>2</sup>, M. Errede<sup>1</sup>, T. Wälchli<sup>3</sup>, K. Frei<sup>3</sup>, R. Perris<sup>3</sup>, L. Roncali<sup>1</sup>

<sup>1</sup>Dept. of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari School of Medicine, Bari; <sup>2</sup>COMT – Centre for Molecular and Translational Oncology & Dept. of Biosciences, University of Parma, Parma, Italy; <sup>3</sup>Dept. of Neurosurgery, University Hospital Zurich, CH-8091 Zurich, Switzerland

NG2/CSPG4 is a complex surface-associated proteoglycan (PG) recognized to be a prognostic marker of glioblastoma multiforme (GBM) and a critical membrane component of angiogenic pericytes. To determine whether GBM cells and pericytes may express different isoforms of the PG we have taken advantage of a recently generated panel of 63 monoclonal antibodies (mAbs) raised against human NG2/CSPG4 to perform a distributional mapping of the PG in human fetal and adult brain and GBM. A total of 48 mAbs were selected on the basis of a repertoire of immuno-assay and were employed for immunohistochemical detection of putative tumor-associated NG2/CSPG4 isoforms. 14 isoforms, revealed *in situ* by the corresponding mAbs, were classified in four distinct groups according to their immunohistochemical distribution pattern, and a prototype clone was selected for each group and extensively used in the study. In adult brain, NG2/CSPG4 appeared poorly represented, whereas isoforms detected by mAbs 2164H5, 2161F9, and 2161D7 were abundant in foetal brain and GBM lesions. In particular, clone 2164H5 identified cell surface shed NG2/CSPG4 fragments included in the ECM, immunolabellings performed with mAb 2161F9 or 2161D7 revealed pericyte-specific isoforms, whereas clone 2166G4 was found to be specific for tumor cells being absent on foetal, angiogenic pericytes. An additional NG2/CSPG4 isoform recognized by clone 2161F9 was uniquely expressed by pericyte precursor cells (PPCs) present in the tumor vessel lumen, while several of the other mAbs reacted with cell surface shed fragments of the PG. The results demonstrate a developmentally regulated modulation of NG2/CSPG4 expression in the human CNS and highlight an unprecedented cell- and tissue-specific isoform variation in foetal brain and GBM. The existence of diverse NG2/CSPG4 isoforms could be related to potential structural-functional variations of the PG and may open new avenues for differential immunotherapeutic targeting of tumorigenic processes.

## THE ISOTROPIC FRACTIONATOR AS A TOOL FOR QUANTITATIVE ANALYSIS IN CNS DISEASE

I.E. Repetto<sup>1</sup>, R. Monti<sup>1</sup>, R. Lent<sup>2</sup>, C.H. Andrade-Moraes<sup>2</sup>, A. Vercelli<sup>1</sup>

<sup>1</sup>Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Italy; <sup>2</sup>Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Brazil

The Isotropic Fractionator method was recently designed by Lent and coworkers to overcome methodological and time limitations of standard cell counting techniques (Herculano-Houzel *et al*, 2005). By this method a basically anisotropic brain or any other region of interest is rendered isotropic through homogenization which breaks the cell but not the nuclear membrane. The suspension obtained is stained for DNA and immunostained for the neuronal-specific antigen NeuN. Aliquots from this suspension can be observed into a Neubauer's chamber, and the density of nuclei of all cells and neurons, investigated under a fluorescence microscope. The whole number of cells/neurons results from the product of nuclear density for the suspension volume. Here we decided to apply this method in counting the number of cells in the CNS in pathological conditions, in order to evaluate cell loss and eventually the neuroprotective effects of drugs. We obtained results on the whole number of neurons in the rat brain similar to those obtained by Lent's method thus validating our processing procedure. Sprague-Dawley (SD) rats were lesioned by Tamura's model (Tamura *et al.*, 1981). Both sides, the ipsilateral and the contralateral to the middle cerebral artery occlusion, were processed for isotropic fractionation method and the neuronal cell loss assessed. We found a 6% neuronal loss on ipsilateral vs controlateral side; the absolute number of neuronal cell was significantly decreased on the lesioned side, and with the TTC staining we correlated the volume lesion (15% of total brain volume) to neuronal loss ( $4.6 \times 10^6$  neurons). To further confirm the feasibility of this method on analyzing pathological conditions and testing the opportunity to work on discrete region of interest, SD rats (200-250 g) were i.p. injected with kainic acid (11 mg/kg) to induce epileptic seizures (Spigolon *et al*, 2010). After dissecting the hippocampus from responsive rats we evaluated the neural cell loss observing 16% reduction of neuronal cells in treated rats vs control group. Therefore we suggest that the Isotropic Fractionator method could represent a simple and reliable method to evaluate the effects of experimental lesions mimicking human diseases, and to consider the neuroprotective effects of different treatments.

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## PHILOSOPHICAL APPETIZER

### HAPPY HOUR WITH THE STRULDBRUGGS

M. Bentivoglio

Dept. of Neurological and Movement Sciences, University of Verona, Italy

Aging of the population is the big issue of our times worldwide, and brain aging is a key topic in this context. Despite vulnerability to neurodegenerative and cerebrovascular disease, a wealth of increasing data demonstrates remarkable capabilities of adaptation and plasticity in the aged brain. For how long can this be extended? This presentation is meant at a reflection beyond aging, on the theoretical and challenging issue of the pursuit of immortality. Although we are all aware that death is implicit in the concept of life, immortality is tempting, as witnessed by literature, art, religion, philosophy, etc. What about science and neuroscience in particular? In the PubMed database, the key word "longevity" results in a bit more than 28.000 hits, "aging" about 290.600 hits, "aging brain" about 48.300 hits. The key words "Immortal brain" result in a timid 100 hits, actually mainly referring to immortal cell lines. But also the issue of medicine (*e.g.* pharmacogenomics) creating a world of Struldbuggs or even Methuselah comes up in the scientific literature: could the brain make it in such a world? What can we learn from the Struldbuggs and who were they? Come to this happy hour to discuss the issue!

## SESSION V NEURODEGENERATION, NEUROPROTECTION, NEUROMODULATION

### EXPRESSION OF AUGMENTER OF LIVER REGENERATION (ALR) IN THE CEREBELLAR CORTEX OF ADULT RATS UNDER NORMAL AND EXPERIMENTAL CONDITIONS

L. Lorusso, P. Flace, G. Ciccimarra, G. Laiso, A. Rizzi, R. Cagiano, G. Ambrosi, V. Benagiano

Dept. of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Aldo Moro, Bari, Italy

The augmenter of liver regeneration (ALR) is an enzymatic protein recently detected in neurons. Since in hepatocytes ALR acts as a growth factor and exerts a protective function against apoptosis and oxidative stress, it has been hypothesized that it could play similar roles in neurons. In the present study, we analyzed the expression of ALR immunoreactivity (IR) in the cerebellar cortex of normal adult rats and evaluated whether it is modified in a hypoxia condition deriving from chronic exposure to mild concentration of CO.

**Material and methods.** The rats were exposed during development of nervous system to low concentrations of CO (5-15 ppm). They were sacrificed at age 3-months and their cerebella subjected to LM immunohistochemistry for ALR.

**Results.** In the cortex of control rats, we found different neurons expressing diffuse cytoplasmic IR, including some Purkinje neurons (in the homonymous layer) and granule and large neurons (in the granular layer). Many ALR-positive punctate elements, putative axon-terminals, were also found distributed throughout the cortex. In the cortex of the CO-exposed rats, the number of positive neurons was greater than in the controls. The positive neurons showed a more abundant IR in their cytoplasm. Also the number of positive punctate elements appeared increased.

**Discussion.** The presence of ALR seems to be correlated with environmental conditions in which neurons may be rather than with specific neuronal types. The increased expression of ALR we reported in this study suggests a reaction with a possible protective effect against hypoxia exerted by ALR on cortico-cerebellar neurons. We previously showed that the hypoxia induced in this experimental model affects GABAergic neurons of the cerebellar cortex, determining significant reduction of expression of glutamic acid decarboxylase (GAD), the GABA synthetic enzyme. Further development of this research will be to evaluate whether in the same model the expression of GAD changes after administration of exogenous ALR.

### DNA METHYLATION OF BDNF GENE INDUCED BY ETHANOL

G. Floris, P. Follesa

Dept. of Life and Environmental Sciences, University of Cagliari, Monserrato, Italy

Cerebellar granule cells in culture are vulnerable to excitotoxicity and brain-derived neurotrophic factor (BDNF) can prevent neuronal damage. However, little is known about the molecular mechanisms that induce neuronal cells to respond to these insults and produce growth factors. Neuronal damage induced by ethanol exposure produces morphological, molecular and functional adaptations of the brain that are thought to be responsible for alcohol addiction. BDNF is one of the candidate molecules involved in such mechanisms, and it has been suggest-

ed to play a role in reducing some of the behavioral effects of ethanol. Multiple actions of ethanol on BDNF gene expression and signaling have been described. Nevertheless the considerable complexity within the BDNF gene itself and the multiple mRNAs encoded by up to 9 potential exons is further complicated because of its epigenetic regulation by methylation of BDNF promoters. The aim of this work was to evaluate the effects of ethanol on CpG islands of BDNF exon IX gene in regulating its expression *in vitro*. Rat cerebellar granule cells in culture were exposed to acute ethanol, chronic ethanol or ethanol withdrawal. Our results demonstrate that ethanol exposure increases, in a dose dependent manner, the abundance of BDNF exon IX transcript in the three different treatment conditions. We then tested the ability of acute ethanol to alter exon IX methylation by using MSP PCR. Similarly to the effect induced by the two DNA methylation inhibitors, zebularine and RG-108, exposure of cultured neurons to 100 mM ethanol for 3h significantly increased the unmethylated state of BDNF exon IX that was about 2.5 folds greater than the methylated state. These results provide the first evidence for an alternative way of ethanol to alter BDNF gene expression. Thus, ethanol induced changes in CpG DNA methylation of BDNF gene seems to be an additional mechanism to implement homeostatic protective actions to prevent adverse effects of ethanol. The ability of ethanol to induce up-regulation of BDNF may play a pivotal role and could result from a number of intracellular responses that include the epigenetic mechanism here described.

### INFLUENCE OF EXTERNAL FACTOR AND AGE ON NEUROINFLAMMATION AND NEURODEGENERATION INDUCED BY 3,4 METHYLENEDIOXYMETHAMPHETAMINE (MDMA)

L. Frau<sup>1</sup>, P.F. Porceddu<sup>1</sup>, M. Morelli<sup>1,2,3</sup>

<sup>1</sup>Dept. of Biomedical Sciences, University of Cagliari; <sup>2</sup>CNR Institute of Neuroscience, Cagliari, Italy; <sup>3</sup>Centre of Excellence for Neurobiology of Dependence, Cagliari, Italy

3,4 methylenedioxyamphetamine (MDMA or ecstasy) is a recreational drug used worldwide, by adolescents and young adults. Several evidences have reported that the use of amphetamine-like drugs could produce neuroinflammatory changes and neurotoxicity toward dopaminergic neurons. Moreover, preclinical studies have shown a potentiation of neuroinflammatory effect of MDMA by caffeine and elevated temperature. Since the recreational intake of MDMA frequently occurs in crowded environments like disco, and the use of MDMA is often associated to caffeinated beverages to increase the effect of MDMA and avoid fatigue, the effects of these conditions in humans abuser should be taken into consideration.

To better clarify the role of crowding condition C57BL/6J mice, 12 and 4 weeks old, considered adults and adolescents respectively, were housed 1, 5 and 10 x cage (24x42x15 cm) and treated with MDMA (4x20 mg/kg, i.p.) whereas to investigate the role of caffeine in MDMA-induced toxicity mice were housed 5 x cage and treated with MDMA (4x20mg/kg, i.p.) in combination with caffeine (2x10 mg/kg, i.p.). Immunohistochemistry for CD11b and GFAP, markers of microglia and astroglia, respectively, and tyrosine hydroxylase, marker of dopaminergic neurons was performed.

Both adult and adolescent mice housed 5 and 10 x cage showed a glial response significantly higher as compared to singly housed and vehicle-treated animals. Moreover, both adult and adolescent mice housed 5 and 10 x cage showed a decrease in the number of TH-positive neurons as compared to singly housed mice and vehicle. However, adolescent mice housed 10 x cage showed a lower number of TH-positive neurons as com-

pared to 5 x cage housed animals.

In adult mice, caffeine potentiated microglial and astroglial inflammation induced by MDMA whereas in adolescent only astroglia was potentiated. Neuronal loss induced by MDMA was increased by caffeine in adolescent mice only, without inducing any modification in adults.

The results suggest that adolescence is a critical period for MDMA-induced toxicity and that the external factors as caffeine or crowded environment could increase neurotoxic responses and worsen the risk associated with recreational use of MDMA.

### MANIPULATING THE AGRIN/NEUROTROPYPSIN SYSTEM IMPROVES MUSCLE TROPHISM IN SMA.

M. Boido<sup>1</sup>, E. De Amicis<sup>1</sup>, S. Hettwer<sup>2</sup>, R. Fariello<sup>2</sup>, A. Vercellotti<sup>1</sup>  
<sup>1</sup>Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Torino, TO; <sup>2</sup>Neurotune AG Wagistrasse 27° CH-8952 Schlieren (ZH) CH

Spinal muscular atrophy (SMA) is a fatal paediatric genetic disease, characterized by motoneuron death, leading to progressive amyotrophic paralysis, respiratory failure, and, in more severe cases, to death. SMA is due to the deletion or mutation of the telomeric survival motoneuron gene (SMN1). Its homologous, SMN2 gene, encodes a truncated protein which can modulate SMA severity. Abnormalities at the neuromuscular junction (NMJ) have been reported in SMA, including neurofilament (NF) accumulation at presynaptic terminals, smaller and immature endplates, reduced transmitter release, and finally muscle denervation. We have studied the effects of manipulating the agrin/neurotrophin system, fundamental in NMJ formation, in SMA delta7 mice: agrin is a synaptic organizer, responsible for NMJ development, i.e. for insertion of a nerve terminal, clustering of AChRs, NMJ maturation. We treated SMA and WT mice from birth with a modified 44kD C terminal agrin fragment (CAF) retaining synaptogenic properties (supplied by Neurotune AG) s.c., using vehicle for controls, which were analysed for behaviour, muscle and NMJ histology, and survival. Motor behavior evaluated by righting reflex and tail suspension tests was significantly improved by treatment in SMA mice. Survival was significantly extended in CAF-treated SMA by 2.5 days (percentage of improvement 16.5%; P=0.001, Kaplan-Meier analysis). Histological analysis was performed on P10 mice. Muscle histology was performed on the quadriceps, a proximal muscle early involved in SMA. In H/E stained muscles, CAF treatment increased cross-sectional area of whole muscle (3083940  $\mu\text{m}^2$  in treated SMA vs 2595276  $\mu\text{m}^2$  in control SMA) as well as of single muscle fibers (141.18  $\mu\text{m}^2$  in treated SMA vs 127.39  $\mu\text{m}^2$  control SMA), thus demonstrating reduced muscle atrophy. NMJ morphology was evaluated in whole mount diaphragm preparations: treated SMA mice showed more mature NMJs and a reduced NF accumulation, compared to controls. On the contrary, no differences were found in the number of lumbar motoneurons between treated and control mice.

Therefore, manipulating the agrin/neurotrophin system in SMA has beneficial effects on muscle trophism and NMJ maturation: this agrin-derived biological restores the crosstalk between muscle and motoneurons, delaying muscular/NMJ atrophy, improving motor performance and finally extending survival.

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### DIETARY ESSENTIAL OIL COMPONENTS IN THE PREVENTION OF ISCHEMIA/REPERFUSION-INDUCED TISSUE DAMAGE IN THE RAT CEREBRAL CORTEX

T. Melis, M.P. Serra, M. Boi, L. Poddighe, C. Picci, M. Del Fiocco, G. Carta, E. Murru, S. Lisai, A.R. Sirigu, M. Collu, S. Banni, M. Quartu

Dept. of Biomedical Sciences, University of Cagliari, Monserrato, Italy

The cerebral ischemia/reperfusion (I/R) injury triggers a metabolic stress that in turn activates local and systemic physiological responses aimed at repairing damage. Brain metabolic stress is tightly linked to lipid peripheral dysregulation, leading to cleavage and release of membrane lipid precursors that can be used as indicators of central nervous system pathologies. We have previously shown that plasma lipid metabolic alterations triggered by I/R during carotid endoarterectomy in patients with carotid stenosis are related to the degree and duration of hypoxic event. The endocannabinoid (eCB) system is a key factor in the response to oxidative and inflammatory damage induced by the I/R challenge and eCB plasma levels have been reported to increase in patients with acute ischemic stroke. In this context, the preventive administration of essential oil constituents, known to modulate the production of eCB congeners, such as palmitoylethanolamide and oleoylethanolamide, may exert important anti-inflammatory, analgesic and metabolic activities.

In this study we extend our previous observations on the beneficial effect of dietary *Pistacia lentiscus* L. essential oils during cerebral I/R injury and examine the activity of some essential oils individual components, such as beta-caryophyllene (BCP) and quercetin (QCT), in Wistar rat cerebral cortex. Cerebral I/R was produced by a 30 min bilateral common carotid artery occlusion (BCCAO) followed by 60 min reperfusion. Animals were starved for 12 hours before surgery and 6 hours prior to ischemia BCP and QCT (40 mg/kg/0.45 ml of sunflower oil as vehicle) were administered via gavage.

Different brain areas were analysed for fatty acid changes, for expression of the enzyme cyclooxygenase-2 (COX-2), eCB receptors, peroxisome proliferator-activated receptor (PPAR) alpha, and for enzymatic activity of matrix metalloprotease 9 (MMP9). Our findings indicate that BCP and QCT appear to influence the outcome of BCCAO/R cerebral injury by modulating levels of polyunsaturated fatty acids, expression of COX-2 and CB receptors, the biosynthesis of eCB and congeners and the activity of MMP9. The results obtained will be discussed in light of the possibility to modulate the physiological response to I/R cerebral injury by means of dietary administration essential oil components.

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## VGF PEPTIDES AND PERIPHERAL ACTIVATION OF ENERGY EXPENDITURE

F. D'Amato, C. Cocco, B. Noli, R. Pilleri, G.L. Ferri

*Dept. of Biomedical Science, Cagliari University, Monserrato, Italy*

VGF precursor protein is processed in various neuropeptides, one of these involved in metabolic mechanisms and named TLQP-21, stimulates the autonomic activation of adipose tissue and reduces the early phase of diet-induced obesity in mouse. We raised antisera to short sequences of the VGF precursor, to reveal in immunohistochemistry (IHC) and ELISA several VGF-derived peptides. Plasma samples of type II diabetes (DMII) were taken from patients with different body mass index (BMI): (i) BMI =18-24, (ii) BMI=25-29, (iii) BMI=30-39, in parallel with plasma samples from healthy subjects matched with BMI of each group (n=10 samples/group). Male CD1 mice were fed with standard chow (slim), or with standard chow plus 36% lard (obese) for 16 weeks (n=24 animals/group). For glucose tolerance test, mice (n=12 each group), received glucose *i.p.* injection (3g/kg), and were sacrificed after 120 minutes. White and brown adipose tissues (WAT and BAT, respectively) were extracted for ELISA (n=9 animals/group), while 4 mice/group were perfused for IHC with 4% paraformaldehyde. In human plasma of DM II patients we found a clear decrease of VGF C-t, in parallel with the increase for BMI (i=1100±85, ii=760±60, iii=550±40 pmol/ml, mean±SEM). Using WAT, we found a significant decrease for VGF C-t in obese compared to slim mice (2±0.5 vs. 5±2\*, pmol/g, mean±SEM, \*p<0.03). A similar trend was observed also in BAT, for VGF C-t and other VGF derived peptides (N-t, TLQP, and RVW peptides). Moreover, exclusively in BAT of slim (but not obese) mice, we measured a great increase in VGF C-t, N-t and RVW peptide levels after glucose load (values taken before and after glucose load: 22±7 vs 35±5\*, 18±4 vs. 30±7\* and 70±10 vs. 95±10\*, respectively, pmol/g, means±SEM, \*p<0.02). IHC data indicated a widespread presence of VGF peptides within noradrenergic perikarya and nerve terminals of celiac ganglion and peripheral tissues (WAT, BAT, and pancreas). In conclusion, more than one VGF fragment could be important for peripheral activation of autonomic nervous system.

## SESSION VI NEURONAL INJURY, PREVENTION AND REPAIR

### EFFECTS OF SYSTEMIC DELIVERY OF TWO DIFFERENT DRUGS, METHYLPREDNISOLONE SODIUM SUCCINATE (MPSS) AND ROLIPRAM, AFTER ACUTE SPINAL CORD CONTUSION

S. Raimondo<sup>1</sup>, G. Ronchi<sup>1</sup>, S. Geuna<sup>1</sup>, L.M. Costa<sup>2</sup>, J.E. Pereira<sup>2</sup>, E. Nikulina<sup>3</sup>, M.T. Filbin<sup>3</sup>, A.S.P. Varejão<sup>2</sup>

<sup>1</sup>Neuroscience Institute of the Cavalieri Ottolenghi Foundation and Department of Clinical and Biological Sciences, University of Turin, Turin, Italy; <sup>2</sup>Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal; <sup>3</sup>Biology Department, Hunter College, New York, New York USA

The treatment of acute spinal cord injury (SCI) is a relevant health issue. We report on the results of two studies aimed at evaluating, in a standardized rat experimental model, whether two different drugs (MPSS and Rolipram, respectively) reduce the size of lesion area if systemically administered after traumatic SCI. The use of MPSS in patients is already recommended on the basis of NASCIS-2 and NASCIS-3 international protocols while the selective phosphodiesterase type 4 inhibitor Rolipram is a promising putative therapeutic agent for the treatment of acute SCI. In the first study, in adult female rats, MPSS treatment was compared to a control saline-treated group after applying a moderate contusion injury (200 kdyn) at T10 vertebral level using the PSI Infinite Horizon impactor®. Animals were behaviourally tested using BBB locomotor rating scale, inclined plane test, horizontal ladder, beam walk and 3D analysis of hindlimb motion. At week-7 postoperative, spinal cord damage was assessed by stereology. Results showed that functional recovery was not significantly different in MPSS-treated group compared to saline-treated group. Similarly, stereological analysis did not disclose a positive effect in the MPSS-treated group. In the second study, using the same moderate contusion injury experimental model, we compared two groups of rats that received either rolipram dissolved in DMSO or only DMSO using an osmotic mini-pump. Functional and morphological recovery was assessed using the same methods described for the previous study. Results showed that rolipram administration improved motor performance at each time-point analysed. Stereological analysis that spared white matter was significantly higher in the group treated with rolipram. Taken together results of this study suggest that MPSS does not have a protective effect after acute SCI while rolipram can have therapeutic role.

## THIOCTIC ACID ENANTIOMERS PREVENT OXIDATIVE STRESS ON SPINAL CORD LESIONS CONSEQUENT TO PERIPHERAL NERVE INJURY

D. Tomassoni<sup>1</sup>, L. Di Cesare Mannelli<sup>2</sup>, N.E. Innocent<sup>3</sup>, C. Ghelardini<sup>2</sup>, S.K. Tayebati<sup>3</sup>, F. Amenta<sup>3</sup>

<sup>1</sup>School of Bioscience and Biotechnology, University of Camerino, Camerino; <sup>2</sup>Dept. of Clinical and Preclinical Pharmacology, University of Florence; <sup>3</sup>School of Medicinal and Health Products Sciences, University of Camerino, Italy

Oxidative stress is an imbalance between the production of free radicals and the antioxidant defence mechanisms, potentially leading to tissue damage. Peripheral neuropathies are in general characterized by hyperalgesia and allodynia with alterations of muscular sensitivity and functions with an increase of oxidative stress. The antioxidant, in particular the thioctic acid, has been proposed as a potential therapeutic agent in the treatment or prevention of several pathologies related to oxidative stress. Thioctic acid is a naturally occurring antioxidant existing in two optical isomers (+)- and (-)-thioctic acid and in the racemic form (+/-). The present study assessed if chronic constriction injury (CCI) of sciatic nerve, induced by loose ligation, is accompanied by a spinal cord changes and if thioctic acid enantiomers treatment has a possible protective role.

Loose ligation of the right sciatic nerve was performed in spontaneously hypertensive rats (SHR), used as a model of increased oxidative stress, and in normotensive Wistar-Kyoto rats (WKY) as control group. Animals with sciatic nerve ligation were left untreated or were treated intraperitoneally for 14 days with (+/-)-(250 µmol/kg/day and 125 µmol/kg/day), (+)-(125 µmol/kg/day), (-)-(125 µmol/kg/day) thioctic acid. Treatment with thioctic acid was compared with pregabalin (300 µmol/kg/day). Analysis was centered on injury phenomena at level of dorsal root ganglia and spinal cord with immunohistochemical and immunohistochemical analysis.

In the spinal cord an increase of oxidative stress marker were observed in rats after sciatic nerve ligation accompanied with an phenomena of astrogliosis and neuronal damage in particular in the posterior dorsal horn without activation of apoptotic process. Treatment with the antioxidant reduced oxidative stress and astrogliosis in spinal cord, in particular (+)-thioctic acid and the higher dose of was (+/-)-thioctic acid active. No effective the (-)-thioctic acid and the pregabalin.

The above results demonstrated a damage in the spinal cord after peripheral nerve injury and neuroprotective effect elicited by thioctic acid. These findings suggest a neuroprotective activity of thioctic acid on central nervous system lesions consequent to CCI and that the compound may represent a therapeutic option for entrapment neuropathies.

## IN VITRO STUDIES ON BIOMATERIALS: PERSPECTIVE OF THEIR EMPLOYMENT IN PROMOTING PERIPHERAL NERVE REGENERATION

F. Fregnan<sup>1,2</sup>, S. Raimondo<sup>1,2</sup>, E. Ciglieri<sup>1,2</sup>, D. Pascal<sup>1</sup>, I. Perroteau<sup>1</sup>, G. Gambarotta<sup>1</sup>, S. Geuna<sup>1,2</sup>

<sup>1</sup>Dept. of Clinical and Biological Science, University of Turin, Orbassano (TO); <sup>2</sup>Neuroscience Institute of the "Cavaliere Ottolenghi" Foundation (NICO), University of Turin, Orbassano (TO), Italy

Depending on the level of severity of nerve injuries the medical treatments to improve nerve regeneration are different. For large nerve gaps, in which end-to-end suture would generate excessive tension, it is necessary to proceed with a tubulization so that the fibers of the proximal stump can regenerate along it

and reaching the distal stump. The practice of autologous grafting has some disadvantages: requires an additional surgical incision for the removal of the healthy sensory nerve, in addition withdrawal of healthy nerve leads to a residue sensory deficit. The new knowledge in tissue engineering, in the possibility of realizing materials for autotransplantation prepared in vitro and in the technology of biomaterials open new interesting perspectives for the realization of innovative and more effective types of graft for the repair of peripheral nerves. Here we propose a study that describes the techniques employed in vitro to assess the compatibility of biomaterials and cell models; important parameters such as proliferation, survival, adhesion, morphology will be taken into account in determining the biocompatibility and the structure more appropriate to the future use of biomaterials in vivo and cellular models are represented by cultures of Schwann cells and explants of dorsal root ganglia.

In particular we detail a study on the chitosan which is obtained from full or partial N-deacetylation of chitin; given the fragility of this material, it has to undergo chemical crosslinking, and we analyzed two substances: dibasic sodium phosphate (DSP), alone or in association with the -glycidoxypropyltrimethoxysilane (GPTMS/DSP). The permissiveness of Chitosan crosslinked with GPTMS / DSP towards important phenomena in nerve regeneration as cell motility and axonal elongation, make desirable to the transfer of the analysis *in vivo*, to test the effectiveness of the material in the promotion of functional recovery of the injured nerve.

## TUBULIZATION TECHNIQUES FOR PERIPHERAL NERVES REPAIR

G. Ronchi<sup>1</sup>, S. Raimondo<sup>1</sup>, S. Bompasso<sup>1</sup>, I. Ridolfi<sup>1</sup>, P. Tos<sup>2</sup>, M.G. Giacobini-Robecchi<sup>1</sup>, B. Battiston<sup>2</sup>, S. Geuna<sup>1</sup>

<sup>1</sup>Dept. of Clinical and Biological Sciences, University of Torino, San Luigi Gonzaga Hospital, Orbassano, Torino;

<sup>2</sup>Reconstructive Microsurgery Division, C.T.O. Hospital, Torino, Italy

The repair and regeneration of peripheral nerve injuries represent a major research field where clinical application of innovative therapies in regenerative medicine should be sought.

The more used surgical technique of repair is a primary end-to-end repair, while in nerve gap injuries, when tension precludes a primary repair, a nerve autograft is usually used to provide a scaffold for the regenerating nerve.

The possibility of repairing nerve defects by bridging the gap by means of non-nervous tubes has been widely studied, both experimentally and in clinical practice. This surgical approach is usually called tubulization, which consists in bridging a nerve gap by means of a cylinder-shaped tube. This technique offers also the possibility of manipulate in the laboratory different tissues and organs in order to fashion conduits that mimic some important features of the nerve environment; and to enrich biological or synthetic tubes with various elements (substances, either molecules, cells or tissues) that are considered essential for promoting nerve fiber regeneration. Moreover, in the last years, several studies about the employment of biomaterials for tubulization were published.

In this presentation, positive and negative *in vivo* results about the use of different conduits in experimental animals is provided.

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